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VOLUME 7 OCTOBER, 1932 NUMBER 4

THE POLYMERIZATION OF ACETALDEHYDE. II[1].

BY W. H. HATCHER[2] AND MURIEL G. KAY[3]

Abstract

Using acetaldehyde prepared out of contact with oxygen, the specific gravities of this substance and its mixtures with paraldehyde have been determined, and shown to differ from those previously obtained. The following values for specific gravity were obtained :— pure acetaldehyde, 0.7865 (15° C.); pure paraldehyde, 0.9984 (15° C.); metaldehyde, 1.120 and 1.127.

The rates of polymerization of acetaldehyde with and without contact with oxygen have been shown to differ, and the latter values are in agreement with a former study.

Historical

In a recent article by Hatcher and Brodie (3), a study was made of the mechanism of the polymerization of acetaldehyde to paraldehyde, phosphoric acid being used as a catalyst. Their findings indicated the reaction to be trimolecular at low concentrations of catalyst, and the velocity to be directly proportional to the quantity of catalyst present. At the same time they observed the necessity of using only freshly distilled material and that *as soon as possible* after distillation. Samples of acetaldehyde gave slow velocities of polymerization when allowed to stand longer than two hours, although no trace of any polymer could be detected in them.

A more recent publication (4) indicated the rapidity with which gaseous acetaldehyde absorbed a small quantity of oxygen from the atmosphere to give rise to a peroxide. Conant (1, 2) has observed an increase in the velocity of polymerization of butyraldehyde due to traces of the peroxide.

In the light of these results it was decided to prepare acetaldehyde in an oxygen-free atmosphere and repeat some of the experiments of Hatcher and Brodie in order to gauge the effect of catalysts excluding oxygen either free or combined.

A careful examination of the specific gravities of mixtures of acetaldehyde and paraldehyde as found by Pascal and Dupuy (6) required redetermination of these values. This has been done, using oxygen-free acetaldehyde.

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Contribution from the Department of Chemistry, McGill University, Montreal, Canada. Submitted in partial fulfilment of the requirements for the degree of M.Sc.

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Experimental

Preparation of Paraldehyde and Acetaldehyde

Paraldehyde was distilled in the usual way from a commercial sample until a fraction was obtained which boiled at 124° C.

Acetaldehyde was prepared in two stages from commercial paraldehyde and a catalyst—concentrated sulphuric acid. In the first stage fairly pure acetaldehyde was distilled from a flask, through a spiral condenser, and into a collecting flask, the condenser and second flask being surrounded by ice and salt. The second stage was the same except that the final receiver was specially constructed with a tube attached to the bottom so that the acetaldehyde could later be forced out by the pressure of nitrogen in the apparatus. Both distillations were carried out in the absence of oxygen; that is, the apparatus in each case was filled with nitrogen at the beginning, and a small stream of nitrogen was passed through continuously during the process. A carbon dioxide atmosphere was used in two cases, but found to give the same results as nitrogen.

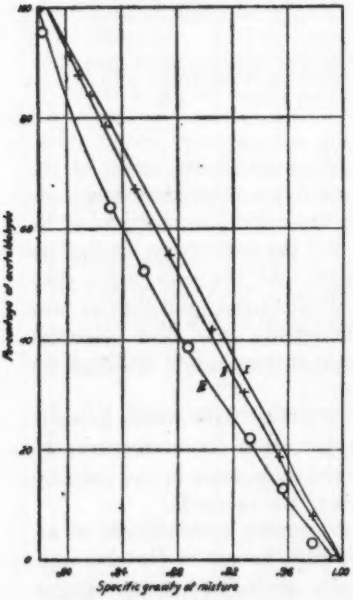

FIG. 1. *Specific gravity curves of acetaldehyde-paraldehyde mixtures.*

In the first stage, the temperature of the water bath around the starting flask was kept at or below 50° C. in order to hasten the distillation, but in the second the temperature was kept at about 30° C.

Cleanliness of all apparatus concerned with the preparation of materials and with subsequent experimentation was strictly observed. Each piece was washed with alcohol, then thoroughly with water, and dried before each experiment.

Determination of Specific Gravities of Mixtures of Acetaldehyde and Paraldehyde

The apparatus used consisted of a pycnometer, constructed in the form of a bulb of about 80-cc. capacity attached to a narrow tube with a stopcock and side arm at the top. A thermostat was used and kept at 15° C.

The specific gravities of pure acetaldehyde and pure paraldehyde were measured by weighing such an amount of material that the level was in the narrow tube. The bulb was then calibrated with water at this level. For mixtures of acetaldehyde and paraldehyde, varying amounts of paraldehyde were weighed into the pycnometer and enough acetaldehyde added so that the total volume would be a previously calculated one. The weight of acetaldehyde put in the bulb was then found. Thus the percentage composition of the mixtures and their specific gravities were determined.

These results are given in Table I and shown graphically in Curve II of Fig. 1.

TABLE I

SPECIFIC GRAVITIES OF ACETALDEHYDE-PARALDEHYDE MIXTURES

Wt. of acetaldehyde, gm.	Wt. of paraldehyde, gm.	% Acetaldehyde	Total volume, cc.	Specific gravity
66.7574	0.0	100.00	84.879	0.7865
62.1869	5.9883	91.22	84.879	0.8032
60.3126	8.3903	87.78	84.879	0.8095
58.2204	11.1673	83.90	84.879	0.8175
55.2129	15.1132	78.51	84.879	0.8285
48.5633	23.5317	67.36	84.879	0.8494
40.9614	33.2090	55.23	84.879	0.8738
32.2622	44.4950	42.03	84.879	0.9042
24.1411	54.6611	30.63	84.879	0.9283
15.2879	65.6268	18.89	84.879	0.9532
6.9656	76.0483	8.40	84.879	0.9781
0.0	84.9150	0.0	85.05	0.9984

I is a straight line drawn between the specific gravities of pure acetaldehyde and pure paraldehyde and represents the ideal curve for the specific gravities of mixtures of the two. III shows the results obtained by Pascal and Dupuy for such mixtures. I and II refer to specific gravities at 15° C., while III refers to specific gravities at 20° C.

Specific Gravity of Metaldehyde

During the preliminary experiments on the polymerization of acetaldehyde, metaldehyde was almost always formed along with paraldehyde. It was therefore decided that since the specific gravity of metaldehyde would be an important factor in studying the rate of polymerization, it should be determined. This was done by weighing about 1 gm. of metaldehyde in a small specific gravity bottle against water for comparison. Since metaldehyde is insoluble in water, the true weight of water displaced was measurable. Two specific gravity determinations were made and the values found to be 1.120 and 1.127.

Polymerization of Acetaldehyde

When these preliminary data had been obtained, acetaldehyde was subjected to polymerization. Above 10° C., paraldehyde is the usual polymer, while below 10° C. quantities of metaldehyde are commonly obtained also. The dilatometer used was the same as that used by Hatcher and Brodie although the scale reading and the total volume were slightly different. One division on the scale (from a Beckmann thermometer) corresponded to 0.0079 cc. and the total measurable capacity of the dilatometer was 26.071 cc.

In each experiment the catalyst was 0.10 cc. of 85% phosphoric acid. In each case the dilatometer was filled with nitrogen before allowing the acetaldehyde to pass into it; it was then filled with acetaldehyde and allowed to stand for a few minutes in the thermostat. The procedure for studying the polymerization was the same as that of Hatcher and Brodie. The rate of polymerization was measured by watching the decrease in volume of the

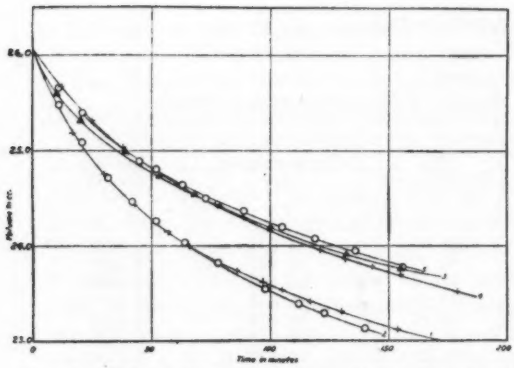

FIG. 2. *Volume-time curves (acetaldehyde-phosphoric acid).*

mixture in the dilatometer, readings of the level of the liquid being taken at intervals of about 10 min.

The results of five typical polymerization experiments are given in Table II and shown graphically in Fig. 2.

From the beginning of the preparation of the acetaldehyde to the end of the polymerization, the work was done with minimum oxygen interference except in cases where oxygen was purposely introduced for a definite length of time.

Curve No. 1 (Fig. 2) is the typical oxygen-free experiment and showed no formation of metaldehyde. No. 2 confirmed No. 1 by coinciding with it up

TABLE II

RESULTS OF FIVE TYPICAL POLYMERIZATION EXPERIMENTS
(0.10 CC. 85% PHOSPHORIC ACID AS CATALYST)

Expt. No.	1	2	3	4	5	Expt. No.	1	2	3	4	5
Time, min.	Total volumes of mixtures, cc.					Time, min.	Total volumes of mixtures, cc.				
0	26.058	26.058	26.058	26.058	26.058	78		23.817			
10	25.453		25.587			79			.418		
11		25.469			25.650	86	.738			*	
12				25.666		89				.291	.355
17	.176					97	.612				
20			.311			98		.541			
21		.081			.390	100			.181	.157	
25				.303		105	.533				.197
30	24.758					112		.391			
32		24.780				117	.415				
38				.034		119					.070
39			24.987			121				23.960	
42		.473				123		.288	*		
43	.426					130	.304				
45					24.884	132			23.904	.857	
52		.252		24.789	.797	136					23.944
53			.734			140		.130			
57	.149	*				143				.778	
63					.647	154	.422				*
64		.031		.600		155			.754	.691	
66	23.999					156					.770
68			.544			167	.035				
73					.497	179				.517	
77				.426							

**This indicates the moment at which metaldehyde appeared.*

to 65 min., then separating from it. This separation coincided with the formation of metaldehyde in No. 2.

Since the oxidation of acetaldehyde after it has been prepared in an oxygen-free atmosphere causes the formation of a peroxide, it was decided to see what effect the passing of oxygen through pure acetaldehyde would have on the rate of polymerization. The results of the typical experiments illustrating this effect are Curves Nos. 3, 4 and 5. In Nos. 3 and 4, oxygen was passed through for one minute, and in No. 5 for five minutes*.

Conant and coworkers, in their work on the pressure polymerization of aliphatic aldehydes, found peroxides to be effective catalysts. It was therefore thought possible that in the above experiments where acetaldehyde peroxide was formed by the addition of oxygen, it acted as a catalyst for polymerization before the phosphoric acid was added; and that the rate at the beginning (the fastest) was not being measured, and this could explain why the experiments with oxygen seemed to progress more slowly than those without. This explanation was subjected to proof by almost filling the dilatometer with pure acetaldehyde and then, when the liquid had reached a constant level, filling the dilatometer to the desired level with oxygenated acetaldehyde. No volume change was observed during a period of over an hour.

TABLE III

POLYMERIZATION OF ACETALDEHYDE WITH 0.10 CC. OF 85% PHOSPHORIC ACID AS CATALYST. CALCULATION OF BIMOLECULAR AND TRIMOLECULAR CONSTANTS

Time, min.	Volume, cc.	Density, gm./cc.	Acetaldehyde, %	Wt. of acetaldehyde, gm.	Acetaldehyde, gm. mol./litre	Bimolecular const. $K\times10^4$	Trimolecular const. $K\times10^4$
Curve No. 1							
0	25.958	0.7865	100.00	20.42	17.87		
10	.353	0.8054	90.18	18.41	16.50	4.645	2.708
20	24.966	0.8177	83.84	17.12	15.58	4.113	2.471
30	.658	0.8279	78.58	16.04	14.79	3.887	2.402
40	.392	0.8371	73.88	15.09	14.06	3.791	2.410
50	.185	0.8441	70.39	14.37	13.51	3.613	2.349
60	.000	0.8507	66.96	13.68	12.94	3.551	2.365
70	23.838	0.8565	64.14	13.10	12.48	3.451	2.347
80	.710	0.8612	61.83	12.63	12.10	3.336	2.312
90	.589	0.8654	59.80	12.21	11.76	3.230	2.277
100	.480	0.8696	57.78	11.80	11.42	3.161	2.270
Curve No. 2							
70	23.833	0.8567	64.02	13.07	12.47	3.464	2.360
80	.685	0.8618	61.56	12.57	12.06	3.371	2.342
90	.546	0.8670	59.00	12.05	11.62	3.344	2.372
100	.425	0.8714	56.94	11.62	11.27	3.275	2.369

**The numerical results of Table II and Fig. 2 include the 0.10 cc. of catalyst which was present in the dilatometer.*

From the volume changes, the bimolecular and trimolecular constants were calculated for No. 1 from 10 to 100 min. and for No. 2 after it diverged from No. 1, that is, from 70 to 100 min. These calculations are shown in Table III. Here the volumes referred to do not include the catalyst.

The following method of calculation was used:— as the volume of the mixture at any time was recorded, and as the total weight was constant, the specific gravity of the mixture could be found. From Fig. 1 the percentage by weight of acetaldehyde was obtained, and from this was found the weight of acetaldehyde present, which, with the total volume of the mixture, gave the concentration of acetaldehyde in gram molecules per litre. This value was $(a-x)$ and the concentration of acetaldehyde at the beginning was (a).

The bimolecular constant was then calculated from the equation

$$K = \frac{1}{t} \times \frac{x}{a(a-x)},$$

and the trimolecular constant from

$$K = \frac{1}{2t} \times \left(\frac{1}{(a-x)^2} - \frac{1}{a^2}\right),$$

where t is the time expressed in minutes.

In order to account for the fact that as the volume of the mixture decreases, the concentration of catalyst increases, the value $(a-x)$ was multiplied by the initial volume and divided by the volume of the mixture at the time represented. This was done for four different values of No. 1, and the trimolecular constants calculated from the new values of $(a-x)$. These constants are shown in Table IV beside the corresponding uncorrected values for purposes of comparison.

TABLE IV

CORRECTED AND UNCORRECTED TRIMOLECULAR CONSTANTS

Time, min.	Without correction	Time, min.	With correction
	Trimolecular constant, $K \times 10^4$		Trimolecular constant, $K \times 10^4$
20	2.471	20	1.708
40	2.410	40	1.670
60	2.365	60	1.638
80	2.312	80	1.603

Discussion of Results

The specific gravity of pure acetaldehyde, as seen from Table I, was found to be 0.7865 at 15° C., and the value at this temperature, as calculated from the value at 18° C. given by Smits and de Leeuw (7) is 0.7870. Since the impurities most likely to be in acetaldehyde are acetaldehyde peroxide and paraldehyde, and since these are both heavier than acetaldehyde itself, a lower specific gravity than that before recorded is acceptable.

The specific gravity of pure paraldehyde, as seen in Table I, was found to be 0.9984 at 15° C., while the value recorded by Kekulé and Zincke (5) is 0.9980. The agreement in the case of paraldehyde is good, and, although the same

is approximately true in the case of acetaldehyde, the impurities which can be found in the latter substance are such as to increase its specific gravity.

In order to compare easily the values obtained for specific gravities of mixtures of acetaldehyde and paraldehyde with those obtained by Pascal and Dupuy, three graphs were plotted in Fig. 1. No. I is the ideal curve and would be the true one if there were no shrinkage of volume when acetaldehyde and paraldehyde are mixed; II represents the experimental data of Table I; III shows the values obtained by Pascal and Dupuy—done, however, at 20° C. It is seen from Fig. 1 that II is much closer to an ideal straight line drawn between the two ends of the curve than III is. Also the results from which II was plotted are considerably more regular and uniform. This difference may be due to the effect of oxygen.

The specific gravity of metaldehyde is here given for the first time, as a search of the literature reveals no previous value. The values found are 1.120 and 1.127.

The effect of metaldehyde appearing in the dilatometer was, as seen from Curves Nos. 1 and 2 of Fig. 2, to produce a larger contraction in volume and therefore make the rate appear faster than if only paraldehyde were formed. This same effect is observed by comparing No. 3 with No. 4, both of which were experiments in which oxygen was passed through the acetaldehyde for one minute after preparation. The time when metaldehyde was first seen in No. 4 practically coincides with the point where No. 4 crosses No. 3 and continues below it, indicating a faster decrease in volume. This is due to the fact that the specific gravity of metaldehyde is appreciably greater than that of paraldehyde—the first polymer to be formed.

In Table III are seen both the bimolecular and trimolecular constants calculated for No. 1 at 10-min. intervals up to 100 min., and for No. 2 from 70 to 100 min. As in Hatcher and Brodie's work, the trimolecular constants are more consistent and therefore represent more truly the order of the reaction.

Table IV shows a few of the trimolecular constants before and after a correction has been made because of the increasing concentration of catalyst. It is seen that the consistency of the constants is about the same in both cases; that is, the increasing concentration of acid is not an important factor at this concentration since the reaction does not proceed fast enough to make an appreciable difference in the concentration of the acid.

Again, in Fig. 2 is seen the effect of passing oxygen through pure acetaldehyde just before it is used for polymerization. Curves Nos. 3, 4 and 5 are decidedly above No. 1, the standard oxygen-free experiment, and also they are very close to each other. It was shown that the peroxide formed is not alone a catalyst, and in the presence of phosphoric acid it is seen to be a negative catalyst. That is, the general effect of the oxidation of acetaldehyde after it has been prepared free from oxygen is a retardation of the rate of polymerization, and the amount of oxygen passed through seems to be immaterial. This fact is of interest since these data are the reverse of those obtained by Conant and coworkers using butyraldehyde. However, the conditions of their experiments are materially different from those obtaining here.

This work is being extended to ascertain whether these results permit of greater generalization. In addition to the new data provided, these findings indicate the necessity of preparation and use of aldehydes out of contact with oxygen.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XLI. STRUCTURE OF RAFFINOSE LEVAN[1]

By Walter Mitchell[2] and Harold Hibbert[3]

Abstract

Raffinose levan has been synthesized by the action of *B. subtilis* on raffinose. The product is identical in structure with sucrose levan previously synthesized by the action of *B. subtilis* and *B. mesentericus* respectively on sucrose, and thus represents a polymerized 2:6-anhydro-fructofuranose. The work serves to confirm the theory put forward previously to the effect that levan formation takes place only with sugars containing a terminal fructofuranose grouping. *B. subtilis* and *B. mesentericus* thus exert quite a specific action with respect to the synthesis of levan.

Introduction

In previous communications dealing with the preparation and properties of sucrose levan (1, 2, 3) it has been shown that, irrespective of whether levan is formed from sucrose by *B. subtilis* or *B. mesentericus*, the resulting product in each case is a polymerized 2:6-anhydro-fructofuranose.

In the first communication on this subject (1), the opinion was expressed that, in all probability, with each of these organisms levan formation takes place only with sugars containing a terminal fructofuranose group as, for example, with sucrose, raffinose, turanose, gentianose and stachyose.

In the case of melezitose, which represents a trisaccharide containing a centrally situated, and not a terminal, fructofuranose linkage, no levan formation was found to take place.

In the present communication it is shown that levan prepared by the action of *B. subtilis* on raffinose is identical with that prepared from sucrose, and is therefore a polymerized 2:6 fructofuranose.

Further investigations are under way regarding the structure of "turanose levan" and "gentianose levan".

In view of the remarkable interest attaching to polysaccharides from the point of view of their immunological action this phase of the work is to be investigated by Dr. FitzGerald and coworkers at the University of Toronto.

Experimental

Synthesis of Raffinose Levan

The procedure used in this work is essentially that used by Harrison, Tarr and Hibbert (1). Fifteen per cent raffinose broth was employed, this concentration representing the maximum solubility of the sugar. The yield obtained is much lower than that from sucrose, which can be partly accounted for by the fact that raffinose is a trisaccharide. From 125 gm. of raffinose 11 gm. crude product was obtained, and from the latter about 50% of purified material.

[1] *Manuscript received August 31, 1932.*
Contribution from the Department of Industrial and Cellulose Chemistry, McGill University, Montreal, with financial assistance from the National Research Council of Canada.
[2] *Research Chemist, Division of Industrial and Cellulose Chemistry, McGill University, Montreal.*
[3] *Professor of Industrial and Cellulose Chemistry, McGill University, Montreal.*

The method of purification is given below. The yield of pure levan is only about 4.5% or approximately 15% of the fructose fraction. It should be noted, however, that the strain of *B. subtilis* employed continued to show an increasing activity towards raffinose, the last yield obtained being 22%, calculated on the fructose fraction. The unchanged raffinose left in the mother liquor was recovered by evaporation, purified by recrystallization and re-used.

Owing to the rarity of raffinose as compared with sucrose a much smaller amount of material was at the authors' disposal, and some slight modifications in technique had, therefore, to be introduced, in dealing with the raffinose levan. Preliminary experiments with small quantities (3 gm. or less) of sucrose levan showed that it was advisable, first, to methylate the levan using dimethyl sulphate and potassium hydroxide, and then to acetylate this partially methylated product and to follow this by a combined deacetylation and methylation process, rather than to prepare first the triacetate and then deacetylate and methylate this compound. The objections to the latter procedure are that the yield of levan triacetate is frequently poor, and the product is insoluble in acetone rendering interaction more difficult during the subsequent deacetylation and methylation processes. The purification of the crude levan was carried out in exactly the same manner as described in a previous communication dealing with sucrose levan (3). A rotation of a sample of the purified raffinose levan dried at 100° C. over phosphorus pentoxide at about 1 mm. showed $\left[a\right]_D^{22} = -45.5°$ in water. An analysis and rotation of the acetate were then made.

Pure white, powdery raffinose levan (0.20 gm.) was added to a mixture of 20 cc. of pure, dry pyridine and 10 cc. of pure acetic anhydride, and the combined products boiled under reflux for about 10 min. A very small amount of a gel remained undissolved and was removed by centrifuging after the mixture had stood overnight at room temperature. The clear solution was poured into about 200 cc. of ice-water and extracted three times with chloroform. After drying over anhydrous magnesium sulphate the solution was concentrated to a gummy residue under 8 mm. pressure, the final temperature reaching 100° C.

The product left was boiled under reflux for two hours with 100 cc. of dry ether. The resulting powdery substance was dissolved in the minimum quantity of methyl acetate and then reprecipitated by dropping the solution slowly into a well-stirred quantity (250 cc.) of dry ether contained in a centrifuge glass. The purified levan triacetate was removed by centrifuging and dried in an Abderhalden vacuum dryer over phosphorus pentoxide at 80° C. Yield, 0.22 gm. $\left[a\right]_D^{20} = +8.9°$ in acetylene tetrachloride (c = 3.01).

Analysis: Found: C, 49.8; H, 5.9%. Calcd. for $(C_{12}H_{16}O_8)_n$: C, 49.98; H, 5.6%.

Methylation of Raffinose Levan

Purified raffinose levan (5 gm.) was dissolved in 5 cc. of distilled water and stirred vigorously whilst 22 cc. of dimethyl sulphate and 44 cc. of 30% potassium hydroxide were added during 2½ hours. Stirring was continued for a further

two hours before diluting the reaction mixture with about 200 cc. of distilled water. The partially methylated levan was extracted with chloroform five times. This extract was dried over anhydrous magnesium sulphate and then concentrated to a semi-solid at 100° C. under 10 mm. pressure. This was dissolved in 30 cc. of pure dry pyridine, 21 cc. of acetic anhydride added, and the solution boiled gently under reflux for five minutes. After standing for five hours the clear solution was poured into about 500 cc. of ice-cold distilled water and extracted three times with chloroform, the product dried and chloroform removed in the usual manner.

The gummy solid so obtained was dissolved in 20 cc. of pure, dry acetone and methylated with 30 cc. of dimethyl sulphate, and 60 cc. of 30% potassium hydroxide added during two hours, with vigorous stirring, the temperature being raised from 50 to 100° C. during the addition.

The reaction product was then treated in exactly the same manner as in the first methylation. The residual solid was purified by precipitation from its solution in methyl acetate with dry ether. It was then redissolved in benzene and reprecipitated with petroleum ether (b.p. 30–50° C.). The pure white powder was dried under reduced pressure over phosphorus pentoxide at 75° C. and 8 mm. for 12 hr. Methoxyl estimation: 20.20 mg. substance gave 66.5 mg. AgI.—OCH_3, 43.5%.

This partially methylated levan was now first acetylated, and then, in one operation, deacetylated and methylated, using the previously described technique. The methoxyl content remained unchanged.

Recourse to the Purdie method was therefore necessary. The solid was dissolved in 50 gm. of methyl iodide and to this vigorously stirred solution was added 30 gm. of dry silver oxide in six five-gram lots at intervals of 30 min. The temperature of the reaction mixture was kept at 0–5° C. during the first two additions. It was then raised to 45–50° C. so that the methyl iodide refluxed gently. The procedure thereafter was exactly as described previously (2). The product was treated with a further quantity of 30 gm. of silver oxide and 50 gm. of methyl iodide. It was then purified by dissolving in benzene and precipitating with petroleum ether (b.p. 60–70° C.). This was repeated twice, using, however, petroleum ether (b.p. 30–50° C.) in both cases. Yield, 4 gm. A sample was dried at 80° C. over phosphorus pentoxide at 8 mm. Analysis: 19.7 mg. gave 65.4 mg. AgI; $-OCH_3$, 44.8%. Calcd. $-OCH_3$ = 45.6%. $[a]_D^{23} = -89.0°$ in tetrachlorethane (c = 2.85).

Hydrolysis of Fully Methylated Raffinose Levan

The procedure followed exactly the description already given by Hibbert and Brauns (2). From 4 gm. of the methylated levan about 1.5 gm. of crystalline 1:3:4 trimethyl fructose was isolated from a light-brownish gum which still continued to show signs of crystallization. M.p. 74° C.; mixed m.p. with authentic specimen, 74° C. Rotation $[a]_D^{25} = +22°$ (c = 3.82).

TABLE I

PROPERTIES OF SUCROSE LEVAN, RAFFINOSE LEVAN AND DERIVATIVES

	Sucrose levan	Raffinose levan
	Properties	
Levan	$[\alpha]_D^{22} = -46.1°$	$[\alpha]_D^{22} = -45.5°$
Levan triacetate	$[\alpha]_D^{24} = +9.0°$ (c = 1.96)	$[\alpha]_D^{20} = +8.9°$ (c = 3.01)
Trimethyl levan	$[\alpha]_D^{22} = -88.0°$	$[\alpha]_D^{23} = -89.0°$
Trimethyl fructofuranose	M.p. 74° C. $[\alpha]_D^{22} = +21.55°$ (c = 3.56)	M.p. 74° C. $[\alpha]_D^{25} = +22°$ (c = 3.82)

Acknowledgment

The authors express their indebtedness to Miss Frances Fowler for the preparation of the crude raffinose levan.

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THE SORPTION OF TANNIC ACID BY PROTEINS AND THE MECHANISM OF VEGETABLE TANNING[1]

By Wilfred Gallay[2]

Abstract

Tannic acid is taken up by gelatin and casein from aqueous solution according to the adsorption isotherm. The temperature coefficient of this sorption is negative. From aqueous alcohol and aqueous acetone solution, the sorption obeys Henry's law. The sorption of tannic acid by hide powder was measured together with the swelling of the latter and the two found to vary in a parallel manner. The same relation was found for the sorption of tannic acid by hide powder with varying alkalinity. The sorption of sodium hydroxide on hide powder obeys the adsorption isotherm. When various acids are used to adjust the system to constant pH, the sorption of tannic acid on hide powder varies essentially as the degree of swelling of the adsorbent. Relations are shown between the viscosities of ethyl alcohol-water mixtures and the sorption of tannic acid on hide powder from corresponding solutions. No lyotropic effect is obtained upon the addition of electrolytes to the sorption system tannin-gelatin. The results obtained are discussed from the point of view of the mechanism of vegetable tanning, and it is concluded that the amount of sorption depends to a great extent upon the degree of swelling of the adsorbent.

In early investigations of the fixation of tannic acid and tannins by proteins the results were considered from the point of view of the law of definite proportions and numerous attempts were made to determine the relative combining weights of tannin and gelatin. No satisfactory explanation of the widely divergent results obtained could be made, and more recent knowledge of the behavior of colloidal solutions renders the idea of direct compound formation between tannin and protein entirely untenable.

Among the several theories and their modifications with respect to the mechanism of tannin fixation, that due to Procter and Wilson has received the most consideration. According to this theory, the protein must first combine with hydrogen ion, thus becoming positively charged, and that it then, upon coming in contact with tannin particles, combines with them through mutual discharge. According to this conception the hydrogen ion concentration is the only important variable factor in the system.

There are several fundamental objections to this theory. There is some doubt whether tannin bears a charge since some electrophoretic measurements have yielded negative results (4). The fact that no coagulation is brought about by tannin in an isoelectric gelatin sol is readily understood from other considerations. It has been amply shown that the stability of a hydrophilic sol depends upon two factors, *viz.*, charge and hydration, of which the latter is by far the more important. The addition of a tannin sol to an isoelectric gelatin sol changes the character of the latter from hydrophilic to hydrophobic, desolvation being effected and the residual stability being now due solely to the electrokinetic potential. The level of the latter may be readily reduced to the critical one by the addition of traces of electrolytes, when precipitation

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immediately occurs (5). Tannic acid fixation on protein in alkaline medium, although much less than in acid, is nevertheless appreciable and this fact is obviously in direct opposition to a theory involving mutual discharge. Wilson (13, pp. 577-578) offers the explanation that this is due to traces of impurities in the tannic acid and to products formed by hydrolysis and oxidation of the tannic acid.

The amount of tannic acid fixed by protein is known to vary with the concentration and in several cases it has been shown that the relation may be expressed by the empirical general parabolic equation (in this connection commonly termed the adsorption isotherm),

$$\frac{x}{m} = \beta C^{\frac{1}{n}},$$

where $\frac{x}{m}$ is the amount adsorbed per unit weight of adsorbent, C is the concentration of adsorbate at equilibrium, and β and $\frac{1}{n}$ are constants. This expresses a condensation of tannin particles on the surface of the adsorbent. The colloidal units of the latter may be looked upon as being agglomerations of condensed and polymeric chains, loosely bound and forming very porous micelles. The constituent fibres of these micelles have been forced apart by imbibition of the solvent. It is difficult to speak of a surface here in the ordinary sense of the word. The outer surface of the micelle forms probably a very small part of the total effective surface. According to the prevailing conditions, the type of adsorbate and of dispersion medium, this surface may or may not have the power of adsorbing a dissolved substance on it. Whether, however, it possesses this power or not, particles dissolved in the medium, if sufficiently small, will be present within the pores of the micelle as well as in the surrounding medium. There will, then, be two phases, one protein-tannin-water and the other tannin-water. In other words, assuming that there takes place no surface adsorption, there will result a simple equilibrium, a partition of the solute between the protein and the water.

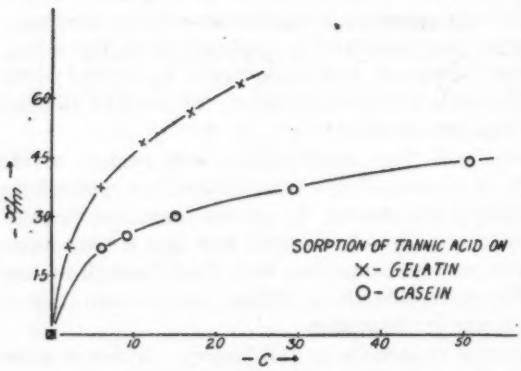

FIG. 1. *Sorption of tannic acid on gelatin and casein.*

Tables VIII and IX and Fig. 1 show the results obtained in the sorption of tannic acid on gelatin and casein, the adsorbents being in aqueous solution before the addition of the adsorbate.

In Fig. 2 the relation between the amount adsorbed and the concentration is logarithmically shown according to

$$\log \frac{x}{m} = \log \beta + \frac{1}{n} \log C.$$

The values of $\frac{1}{n}$ may be directly measured and the mean values of β calculated. From these figures there can then be obtained calculated values of $\frac{x}{m}$. In Tables I and II these values of $\frac{x}{m}$ are compared with the values obtained experimentally. It is seen that the differences are comparatively small and hence the adsorption isotherm is well obeyed over the range measured.

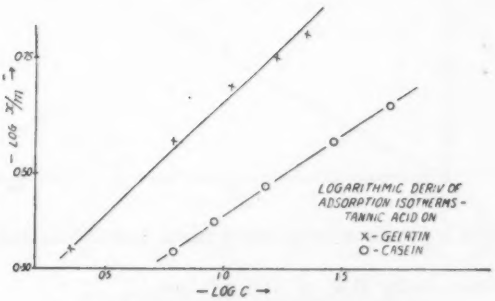

FIG. 2. *Logarithmic derivatives of adsorption isotherms-tannic acid on gelatin and casein.*

Cases of pure adsorption are known. As examples may be cited the adsorption of gold and arsenic trisulphide by carbon and barium sulphate (12), and of carbon suspension by paper (8). However in several instances, investigators have found that adsorption and absorption (solution) occur together. McBain (6) states that hydrogen is partly dissolved and partly

TABLE I

SORPTION OF TANNIC ACID BY GELATIN FROM AQUEOUS SOLUTION

$\frac{1}{n} = 0.448 \quad \beta = 1.603$

C, millimoles per litre	22.96	16.98	11.09	6.15	2.24
$\frac{x}{m}$, calc.	6.52	5.70	4.69	3.62	2.30
$\frac{x}{m}$, obs.	6.35	5.65	4.90	3.75	2.20

TABLE II

SORPTION OF TANNIC ACID BY CASEIN FROM AQUEOUS SOLUTION

$\frac{1}{n} = 0.335 \quad \beta = 1.189$

C, millimoles per litre	51.06	29.56	15.14	9.28	6.20
$\frac{x}{m}$, calc.	4.45	3.70	2.82	2.49	2.19
$\frac{x}{m}$, obs.	4.43	3.71	2.98	2.50	2.18

adsorbed by charcoal. Georgievics (3) came to the same conclusion with dilute acids and charcoal. Davis (1) and McBain (7) found in the sorption of iodine by charcoal that an initial fast adsorption was followed by slow diffusion and solution over a period of years. It is therefore a difficult matter to ascribe to each its individual effect. Adsorption, considered as a measure of the attraction between the adsorbent and solute, is opposed by the mutual attraction between solute and solvent. If then the latter be sufficiently increased, adsorption may be reduced to a minimum. The exponent $\frac{1}{n}$ of the adsorption isotherm finally would reach the value one for zero adsorption, the expression then being that of Henry's law,

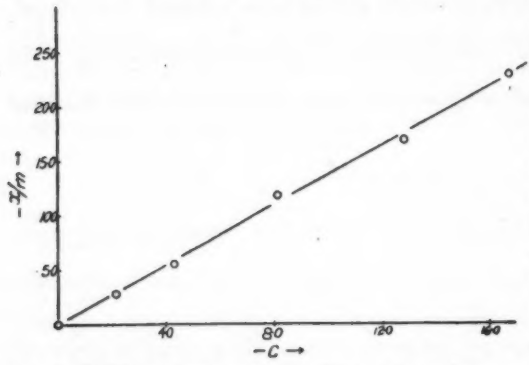

FIG. 3. *Sorption of tannic acid on gelatin in ethyl alcohol-water.*

$$\frac{x}{m} = \beta C.$$

The writer has found this to be true for the case of the sorption of iodine by polyvinyl alcohol (2). This sorption obeys the adsorption isotherm over wide ranges in aqueous solution, but in aqueous alcohol solution, *e.g.*, 75% alcohol, the relation between amount taken up and concentration is expressed by a straight line, a simple partition of the solute between solvent and absorbent. The same has been found in the present instance. The sorptions of tannic acid on gelatin in aqueous alcohol and aqueous acetone solutions obey Henry's law as shown in Tables X and XI and Fig. 3.

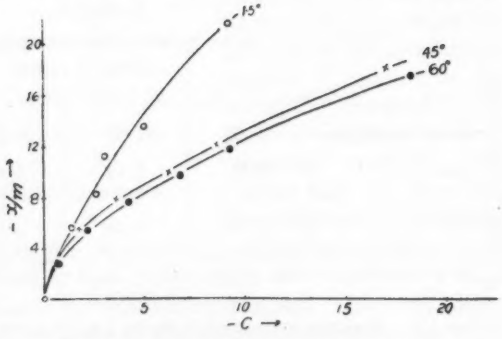

FIG. 4. *Sorption of tannic acid by gelatin.*

The above experiments were carried out at room temperature since it was found that small variations in temperature were without appreciable effect on the extent of sorption. In order to measure the temperature coefficient over a wide range, the sorption of tannic acid on gelatin was carried out at 60°, 45° and 1.5° C. The results are shown in Table XII and Fig. 4.

It will be seen that the temperature coefficient is very appreciable over wide ranges and is negative as in the case of charcoal. The curves measured at 60° and 45° C. are affine ($\frac{1}{n}$ =0.560 and 0.572 respectively), *i.e.*, one is obtained by multiplying the ordinates of the other by a constant factor. From this may be concluded that the true adsorptive power is the same in each case but the specific surface is greater at the lower temperature. On this basis some change in the nature of the surface has taken place at 1.5° C. causing a marked increase in $\frac{1}{n}$. These differences are particularly striking in the logarithmic derivatives shown in Fig. 5.

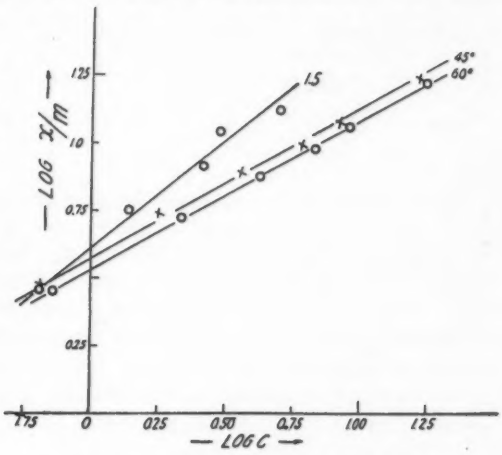

FIG. 5. *Logarithmic derivatives of sorption isotherms—tannic acid on gelatin.*

In Tables III, IV and V are shown the calculated and observed values of x, based on the measured $\frac{1}{n}$ and calculated mean β.

TABLE III

SORPTION OF TANNIC ACID ON GELATIN AT 60° C. (AQUEOUS SOLUTION)

$\frac{1}{n}$ =0.560 β=3.405

C, millimoles per litre	18.13	9.20	6.75	4.23	2.18	0.73
$\frac{x}{m}$, calc.	17.25	11.80	9.92	7.63	5.27	2.85
$\frac{x}{m}$, obs.	17.58	11.84	9.72	7.62	5.34	2.82

TABLE IV

SORPTION OF TANNIC ACID ON GELATIN AT 45° C. (AQUEOUS SOLUTION)

$\frac{1}{n}$ =0.572 β=3.671

C, millimoles per litre	16.90	8.53	6.20	3.60	1.78	0.65
$\frac{x}{m}$, calc.	18.50	12.51	10.42	7.64	5.10	2.87
$\frac{x}{m}$, obs.	18.07	12.11	9.94	7.87	5.50	2.85

TABLE V

SORPTION OF TANNIC ACID ON GELATIN AT 1.5° C. (AQUEOUS SOLUTION)

$\frac{1}{n}=0.885 \quad \beta=0.6287$

C, millimoles per litre	4.98	3.00	2.60	1.38	0.65
$\frac{x}{m}$, calc.	17.60	11.22	9.91	5.63	2.90
$\frac{x}{m}$, obs.	13.53	11.23	8.29	5.66	2.85

The variation in the amount of tannic acid fixed by collagen has been put forth as a basic point of proof for the Procter-Wilson theory (10, 13, Chap. 16). A minimum of tannage is obtained at the isoelectric point of collagen, the amount fixed increasing sharply on the acid side and slowly on the alkaline side. This is interpreted to mean that fixation on the acid side increases due to increased positive charge on the collagen with consequent greater ability to fix tannin through mutual discharge. This matter may be considered, however, from the point of view of specific surface available for sorption. It is well known that various colloidal properties of proteins show a minimum at the isoelectric point, among them being the ability to imbibe solvent. Swelling increases to a maximum with increasing acidity and then decreases, probably due to the dehydrating action of the concentrated acid in the solvent. Since the amount of tannin fixation must be dependent to a great extent upon the specific surface of the protein both for adsorption and solution, a reasonable explanation is at hand for the variation of tannin fixation with pH, without regard to a theory involving mutual discharge.

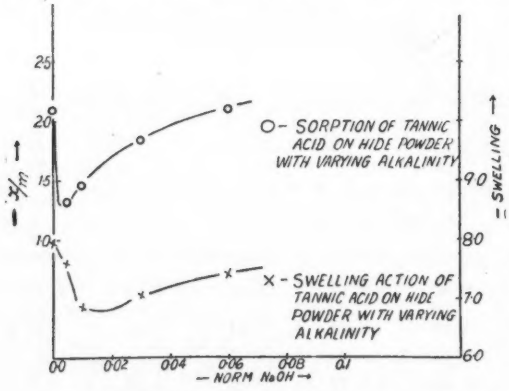

FIG. 6. *Sorption and swelling action of tannic acid on hide powder with varying alkalinity.*

In Table XIII and in Fig. 6 is shown the variation of tannic acid fixation on hide powder with varying pH and constant concentration of solute, and the swelling of each adsorbent at equilibrium. The similarity is striking, the tannic acid fixation appearing to depend directly upon the degree of solvation of the adsorbent.

Table XIV and Fig. 7 show the variation of sodium hydroxide fixation by hide powder with varying concentration. The adsorption isotherm is fairly well obeyed within the range measured as shown by the logarithmic plot of

Fig. 7 and Table VI, where the observed and calculated values of $\frac{x}{m}$ may be compared.

TABLE VI

SORPTION OF SODIUM HYDROXIDE ON HIDE POWDER

$\frac{1}{n} = 0.210 \quad \beta = 0.271$

C, millimoles per litre	101.64	57.23	24.36	3.63	0.53
$\frac{x}{m}$, calc.	0.716	0.635	0.531	0.355	0.237
$\frac{x}{m}$, obs.	0.735	0.629	0.527	0.376	0.215

The sorption of tannic acid on hide powder with increasing concentration of adsorbate follows a different path to those in the cases of gelatin and casein in solution. There is a steep rise (Fig. 8) in the amount taken up until a maximum is reached at an intermediate concentration of tannic acid, followed by a decrease. One explanation given for this course is that with increasing concentration of tannic acid, the rate of combination of the latter with collagen increases so rapidly that soon a point is reached where the surfaces of the protein fibres are so heavily tanned that their permeability is reduced to the remainder of the tannic acid in solution. Another explanation is that of Thomas and Foster (9), who observed that the electrical difference of potential at the surface of tannin particles decreases with increasing concentration of tan liquor. This latter explanation is the one favored by Wilson (13, p. 519), as fitting in with his theory of the mechanism of vegetable tanning. According to this theory, this decrease in potential would lessen the attraction between the tannin particles and the protein gel and thus cause a decrease in the rate of combination.

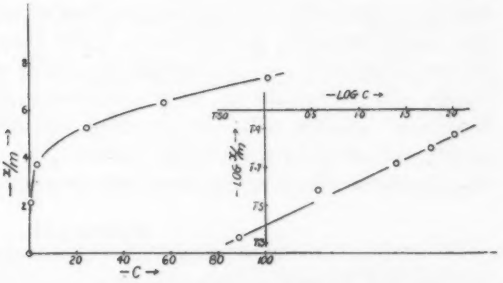

FIG. 7. *Sorption of sodium hydroxide on hide powder.*

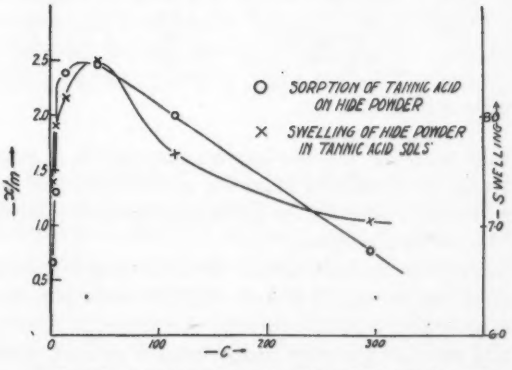

FIG. 8. *Sorption of tannic acid on hide powder and swelling of hide powder in tannic acid sols.*

Table XV and Fig. 8 show the sorption data for tannic acid and hide powder and the corresponding values for the swelling of the adsorbent at equilibrium. As in the case of the series with varying pH discussed above, the fixation of the tannic acid runs parallel to the degree of solvation of the adsorbent.

It must follow directly from the Procter-Wilson theory that at the same pH, no matter how the latter is fixed, the sorption of tannic acid must be constant for the same concentrations of tan liquor. Experiments of Thomas and Kelly (10) however, point to entirely different results. In two parallel sets of experiments in the variation of sorption with concentration of tan liquor, the pH was kept constant at 2 by the addition of acid. In the one case the acid used was hydrochloric and in the other, phosphoric. The fixation in the latter series was by far the greater at equivalent concentrations. At high concentrations of solute the difference amounted to over 100%. The explanation put forth by Wilson (13, p. 574) is that phosphoric acid is a better buffer than hydrochloric, permitting a smaller rise in pH value during tanning or possibly that the increased amount of the weaker acid used to produce the desired pH is responsible. It has been shown that the dissociation constant of the acid used has a great effect on the amount of tannic acid fixed by hide substance (11). The increasing effect of the various acids upon the fixation is essentially the inverse order of the acids arranged according to decreasing dissociation constants. In the present instance a number of sorptions of tannic acid on hide powder at constant pH were measured, the only variation being in the acid used to adjust the system to the desired pH. The swelling of the adsorbent was measured also at equilibrium and the results expressed in Table VII.

TABLE VII

SORPTION OF TANNIC ACID ON HIDE POWDER

Acid used to adjust pH	$\frac{x}{m}$, millimoles per gm.	Swelling of hide powder, per gm.	Acid used to adjust pH	$\frac{x}{m}$, millimoles per gm.	Swelling of hide powder, per gm.
Acetic	2.37	10.1	Oxalic	2.04	7.8
Lactic	2.29	10.2	Tartaric	2.03	9.5
Citric	2.23	9.6	Hydrochloric	1.98	8.4
Phosphoric	2.04	9.9	Sulphuric	1.86	6.6

NOTE: *pH, 2.5; total concentration of tannic acid, 2%.*

The accuracy of the method used for the swelling measurements is comparatively low. However it may be concluded from the general trend of the swelling of the hide powder that the sorption is again shown to be closely related to the degree of swelling of the adsorbent.

A rather significant conclusion may be drawn from the following relations. There is practically no fixation of tannin on hide powder from alcoholic solution. It would be expected then starting with aqueous solution that with solutions of tannic acid in media containing increasingly large proportions of alcohol, there would be obtained a regular decrease of fixation. This however is not found

to be the case (Fig. 9). There appears a well-marked point of change at an alcoholic concentration of about 50% by volume. It is a striking fact that approximately at this concentration does the viscosity-concentration curve of an alcohol-water system show a maximum. The latter is interpreted to mean that compound formation takes place, the relative proportions of alcohol and water in the compound being given by the position of the maximum. On this basis as the percentage of alcohol in the dispersion medium is gradually increased, water is "bound" by the alcohol resulting in a decreased degree of swelling of the hide powder with a corresponding decrease in tannin fixation. Until the point of maximum compound formation is reached, this decrease is rapid and regular. At this point however, that amount of alcohol necessary to "bind" a maximum of water has been reached and the effect of still greater proportion of alcohol in the dispersion medium is simply that due to a decrease in the amount of water necessary for full imbibition by the adsorbent. The decrease after the intermediate point of change is regular but less rapid than the first decrease. Tables XVII and XVIII and Fig. 9 show the results obtained in this sorption series.

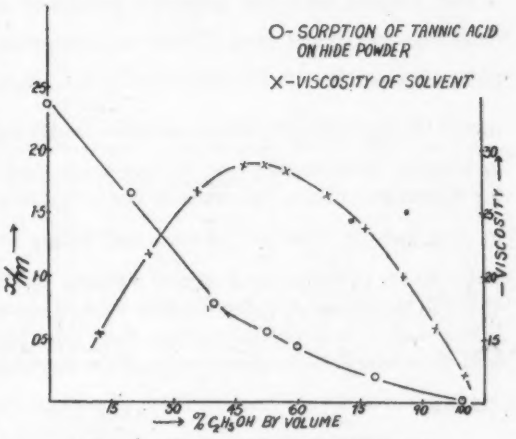

FIG. 9. *Sorption of tannic acid on hide powder and viscosity of solvent.*

The relative effect of equimolecular concentrations of various electrolytes upon the fixation of tannic acid by gelatin was investigated and the results are shown in Table XIX. The additions of electrolyte were made before precipitation in the first series and after precipitation in the second. Appreciable variations in tannin fixation were obtained but in neither series was there observed any lyotropic effects.

Experimental

The tannic acid used throughout the work was "Tannic Acid puriss", of Schuchardt, and solutions were used immediately after preparation. The gelatin sols were not prepared in a standard way and hence, to some extent, only inter-comparisons of results may be made. The hide powder used was the standard material in use in tannin analysis. The Löwenthal oxidation method was used in tannic acid determinations, the procedure being as follows. Standard indigo carmine solution was prepared by dissolving 5 gm. of the dye in 50 cc. of concentrated sulphuric acid and then diluting to one litre with

distilled water and filtering. Potassium permanganate solutions of concentration 0.5 gm. per litre were used, freshly prepared owing to the poor keeping quality of solutions of this strength. To 25 cc. of the dye solution was added 750 cc. of distilled water and an aliquot part of the tannin sol under investigation. The solution was mechanically vigorously stirred during titration with the permanganate. For dilute tannin sols the end-point, denoted by a change to lemon-yellow from blue-green, was fairly sharp, check experiments agreeing within 1%. The dye was standardized alone in terms of the potassium permanganate solution and then tannic acid standardized by using a solution of known concentration. In order to convert to molar concentrations from the weights thus obtained, tannic acid was calculated as $C_{14}H_{10}O_9$.

The amount adsorbed per unit weight of adsorbent, $\frac{x}{m}$, is expressed as millimoles per gram, and C, the concentration of solute at equilibrium, as millimoles per litre. The exponent of the adsorption isotherm, $\frac{1}{n}$, was obtained by plotting $\frac{x}{m}$ and C on a logarithmic diagram, measuring the angle of inclination between the line joining every two experimental points and the log C axis and taking the mean of the tangents. β was obtained by inserting in each case the value of $\frac{1}{n}$ found, and taking the mean.

To 40 cc. of tannic acid sols of varying concentrations, was added 40 cc. of 0.5% gelatin sol and the mixture shaken mechanically for five hours. The gelatin-tannic acid precipitate was then separated by centrifuging and tannic acid determined in an aliquot part of the supernatant liquor.

TABLE VIII

SORPTION OF TANNIC ACID ON GELATIN FROM AQUEOUS SOLUTION

$\frac{1}{n} = 0.448 \quad \beta = 1.603$

Conc. of tannic acid, %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
2.5	1.837	1.269	22.96	6.35
2.0	1.358	1.126	16.98	5.65
1.5	0.887	0.976	11.09	4.90
1.0	0.492	0.750	6.15	3.75
0.5	0.179	0.442	2.24	2.20

In the case of casein, sols of concentration lower than 0.75% left an opalescent solution after precipitation with an equal amount of tannic acid. The procedure was the same as in the case of gelatin.

To 40 cc. of tannic acid sols of varying concentrations was added 40 cc. of 0.5% gelatin sol and the mixtures shaken for one hour. Then 240 cc. of 95% ethyl alcohol was added to each sol. The comparatively highly swollen

precipitates now dehydrated and settled rapidly. The mixtures were shaken mechanically for two hours to equilibrium, and tannic acid determined on an aliquot part of the clear supernatant liquid.

TABLE IX

SORPTION OF TANNIC ACID ON CASEIN FROM AQUEOUS SOLUTION

$\frac{1}{n} = 0.335 \quad \beta = 1.189$

Conc. of tannic acid, %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
4.0	4.085	0.885	51.06	4.43
2.5	2.365	0.741	29.56	3.71
1.5	1.268	0.596	15.14	2.98
1.0	0.742	0.500	9.28	2.50
0.75	0.496	0.436	6.20	2.18

TABLE X

SORPTION OF TANNIC ACID ON GELATIN FROM ALCOHOL-WATER SOLUTION

Conc. of tannic acid, %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
8.0	5.332	4.601	16.66	23.00
6.0	4.069	3.380	12.69	16.90
4.0	2.589	2.378	8.09	11.89
2.0	1.381	1.102	4.31	5.51
1.0	0.687	0.555	2.15	2.78

The above procedure was carried out with acetone in place of alcohol, a longer time being allowed for equilibrium.

TABLE XI

SORPTION OF TANNIC ACID ON GELATIN FROM ACETONE-WATER SOLUTION

Conc. of tannic acid, %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
5.0	5.555	0.653	17.36	3.27
4.0	4.474	0.492	13.98	2.46
3.0	3.385	0.340	10.58	1.70
2.0	2.291	0.192	7.16	0.96
1.0	1.166	0.076	3.64	0.38

To 20 cc. of tannic acid sols of varying concentrations, was added 20 cc. of 0.5% gelatin sol, each at 60° C. The reaction tubes were fitted with capillary outlets and kept in a thermostat at 60° C. for six hours, the contents being stirred from time to time. The mixtures were then filtered through a funnel jacketed by water at 60° C. and tannic acid determined on the filtrates. Two other series were carried out under the same procedure except that the temperatures maintained were 45° and 1.5° C.

TABLE XII

SORPTION OF TANNIC ACID ON GELATIN AT VARYING TEMPERATURES

	Conc. of tannic acid %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
t =60° C.	4.0	0.725	1.760	18.13	17.58
	2.5	0.368	1.187	9.20	11.84
$\frac{1}{n}$ =0.560	2.0	0.270	0.973	6.75	9.72
	1.5	0.169	0.764	4.23	7.62
β =3.405	1.0	0.087	0.534	2.18	5.34
	0.5	0.029	0.282	0.73	2.82
t =45° C.	4.0	0.676	1.807	16.90	18.07
	2.5	0.341	1.211	8.53	12.11
$\frac{1}{n}$ =0.572	2.0	0.248	0.994	6.20	9.94
	1.5	0.144	0.787	3.60	7.87
β =3.671	1.0	0.071	0.550	1.78	5.50
	0.5	0.026	0.285	0.65	2.85
t =1.5° C.	2.5	0.199	1.356	4.975	13.53
	2.0	0.120	1.123	3.000	11.23
$\frac{1}{n}$ =0.885	1.5	0.104	0.829	2.600	8.29
	1.0	0.055	0.566	1.375	5.66
β =4.253	0.5	0.026	0.285	0.650	2.85

To 100 cc. of 2% tannic acid sols to which varying amounts of sodium hydroxide had been added, were added 2 gm. of hide powder and the mixtures shaken mechanically for five hours and allowed to stand overnight. The mixtures were then filtered, using short stem filter funnels, through cotton which had previously been wet and allowed to drain. All filtrations were made in as standard a way as possible. The receivers were 100-cc. volumetric flasks, and after filtration were filled to the mark with water from a burette, compensating for the water retained by the hide powder. Tannic acid determinations were made in the manner already described. Hydrolysis of the hide substance was marked at the highest concentration of sodium hydroxide.

TABLE XIII

SORPTION OF TANNIC ACID ON HIDE POWDER WITH VARYING ALKALINITY

Sodium hydroxide added, gm.	Tannic acid left in solution, millimoles	Tannic acid fixed, millimoles	$\frac{x}{m}$	Swelling per gm.
0	2.022	4.188	2.09	7.95
0.02	3.576	2.634	1.32	7.60
0.04	3.288	2.922	1.46	6.85
0.12	2.524	3.686	1.84	7.05
0.24	1.987	4.223	2.11	7.40
0.40	—	—	—	—

To 100 cc. of sodium hydroxide solutions of varying concentrations was added 2 gm. of hide powder and after mechanical shaking for five hours the mixtures were centrifuged and sodium hydroxide was determined in the clear liquid.

TABLE XIV

SORPTION OF SODIUM HYDROXIDE ON HIDE POWER

$\frac{1}{n} = 0.210 \quad \beta = 0.271$

Normality of sodium hydroxide	Sodium hydroxide left in soln., millimoles	Sodium hydroxide fixed, millimoles	C	$\frac{x}{m}$
0.1163	10.164	1.470	101.64	0.735
0.0698	5.723	1.257	57.23	0.629
0.0349	2.436	1.054	24.36	0.527
0.0116	0.363	0.751	3.63	0.376
0.0058	0.053	0.429	0.53	0.215

Lots of 2 gm. of hide powder were shaken mechanically for five hours with 100 cc. of tannic acid sols of varying concentrations and then the mixtures allowed to stand overnight. Determinations of the swelling of the hide powder were made in the manner described above and tannic acid was determined in each filtrate.

TABLE XV

SORPTION OF TANNIC ACID ON HIDE POWDER

Conc. of tannic acid %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$	Swelling per gm.
10.0	29.520	1.540	295.20	0.77	7.05
5.0	11.521	4.009	115.21	2.00	7.65
3.0	4.401	4.917	44.01	2.46	8.50
2.0	1.444	4.768	14.44	2.38	8.15
1.0	0.516	2.590	5.16	1.30	7.90
0.5	0.238	1.315	2.38	0.66	7.40

Table XVI shows the amounts of acids used per litre pre-calculated in order that when diluted to twice the volume with 4% tannic acid solution, the final pH obtained be approximately 2.5. The pH was measured by means of a comparator, methyl violet (range 1.8 to 3.2) serving as indicator in all cases.

TABLE XVI

VARIOUS ACIDS USED AND ADJUSTED pH

Acid	Amount used per litre	pH after addition of tannic acid	Acid	Amount used per litre	pH after addition of tannic acid
Hydrochloric	0.53 cc.	2.5	Oxalic	0.832 gm.	2.4
Sulphuric	0.30 cc.	2.5	Lactic	10.81 cc.	2.4
Phosphoric	0.47 cc.	2.4	Tartaric	4.50 gm.	2.4
Acetic	80.0 cc.	2.4	Citric	6.72 gm.	2.4

Lots of 2 gm. of hide powder were shaken mechanically for five hours with 100 cc. of the above acid-tannic acid mixtures and then allowed to stand one

week, after which swelling and tannic acid determinations were made in the usual manner. The results are expressed in Table VII.

Tannic acid solutions (2%) were prepared in media containing varying proportions of 95% ethyl alcohol. Lots of 2 gm. of hide powder were shaken mechanically for six hours with 100 cc. of these solutions and allowed to stand overnight after which tannic acid determinations were made.

TABLE XVII

SORPTION OF TANNIC ACID ON HIDE POWDER FROM WATER-ALCOHOL SOLUTIONS

Ethyl alcohol, % by volume	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	$\frac{x}{m}$
100	6.154	0.054	0.027
80	5.804	0.404	0.202
60	5.312	0.896	0.448
50	5.054	1.154	0.577
40	4.631	1.577	0.789
20	2.877	3.331	1.666
0	1.473	4.735	2.368

The following relations between water-alcohol percentage by weight and viscosity of the mixture were taken from the International Critical Tables. The percentage by volume has been inserted for purposes of comparison with Table XVII.

TABLE XVIII

VISCOSITY OF WATER-ETHYL ALCOHOL MIXTURES

Ethyl alcohol, % by weight	Ethyl alcohol, % by volume	Viscosity (poises)	Ethyl alcohol, % by weight	Ethyl alcohol, % by volume	Viscosity (poises)
10	12.39	15.5	60	67.69	26.4
20	24.47	21.7	70	76.91	23.7
30	36.18	26.7	80	85.46	20.0
40	47.33	28.7	90	93.25	16.0
45	52.66	28.7	100	100.00	12.2
50	57.83	28.3			

TABLE XIX

EFFECT OF ELECTROLYTES ON TANNIC ACID SORPTION BY GELATIN

Series	Electrolyte	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
A	NaCl	2.104	1.002	6.58	5.00
	NaBr	2.464	0.642	7.70	3.20
	$NaNO_3$	2.415	0.691	7.55	3.45
	Na_2SO_4	2.355	0.751	7.36	3.75
B	NaCl	1.888	1.218	5.90	6.10
	NaBr	1.965	1.141	6.14	5.70
	$NaNO_3$	1.926	1.181	6.02	5.90
	Na_2SO_4	2.003	1.103	6.26	5.50

A. Gelatin sol (40 cc. of 0.5%) was added to 40 cc. of 2.5% tannic acid sol in each of four flasks and after mechanical shaking for two hours, there was added to each 240 cc. of inorganic salt solutions of such concentration, that the salt was present in molar solution in the whole mixture. After five hours of further shaking, the mixtures were centrifuged and tannic acid determinations made.

B. A second series was then carried out with the same procedure except that the electrolyte was added to the gelatin sol before precipitation by tannic acid.

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CHEESE-RIPENING STUDIES[1]

Nitrogen Requirements of Lactic Acid Bacteria

I. THE FRACTIONAL ANALYSIS OF VARIOUS NITROGEN SOURCES USED FOR THE QUANTITATIVE DETERMINATION OF THE SUGAR-FERMENTING ABILITIES OF LACTIC ACID BACTERIA

By Blythe Alfred Eagles[2] and Wilfrid Sadler[3]

Abstract

A study is being made of the nitrogen requirements of lactic acid bacteria. Employing the method of Wasteneys and Borsook, the nitrogen distribution has been determined in nitrogen sources available commercially and in sources that may be readily prepared by laboratory workers. Forty-three sources have been analyzed. The results of the analyses show that peptic casein digest broth contains from 55 to 63% protein nitrogen, 19 to 25% peptone nitrogen, and 14 to 17% subpeptone nitrogen, according to the particular casein used—when the standard method of preparation is followed. If less casein is used for digestion, or if the period of digestion is reduced, the total amount of nitrogen made available is lower; this being true for the subpeptone nitrogen fraction in particular. In tryptic casein digest broth, 70% of the nitrogen is in the subpeptone nitrogen fraction, and about 28% is found as peptone nitrogen. The broth prepared from one commercial source presents a nitrogen distribution picture that is something of a composite of the nitrogen distribution in the standard casein digest and the tryptic casein digest.

When the nitrogen sources fractionated are employed as the substrate for fermentation studies, it will be seen that whilst the suitability of a source is not always fully indicated by the nitrogen distribution picture, the biological significance of the nitrogen distribution in the sources is, on the whole, reflected in the influence on the sugar-fermenting abilities of the lactic acid bacteria reported upon.

For the quantitative determination of the sugar-fermenting abilities of the lactic acid bacteria, Orla-Jensen (2) has shown the importance of employing a suitable nitrogen source. His findings have assumed an added significance as the present authors have worked on the bacteria isolated from ripening Kingston cheese. Supplying nitrogen sources comparable with the breakdown products defined in cheese, they have investigated the influence of these sources on the sugar-fermenting abilities of organisms isolated. It was first necessary to determine the nitrogen distribution in nitrogen sources available commercially, and in sources that may be readily prepared by laboratory workers. Where the material has been prepared by the authors they have described precisely the mode of preparation: where the source is of commercial origin the authors have retained the name used for the product by the manufacturer.

For the nitrogen distribution determinations the method of Wasteneys and Borsook has been employed (1, 6).

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Experimental

Definition and Description of Nitrogen Sources Submitted to Analysis*

NUMBER

1 Peptic casein digest broth prepared after the manner of Orla-Jensen (2, 3) from commercial casein. To 3000 cc. of tap water are added 280 gm. of commercial casein (Will Corporation), 16 gm. of pepsin and 36 cc. of concentrated hydrochloric acid — Orla-Jensen uses 8 gm. of pepsin. With frequent shaking the whole is allowed to digest for 10 days at 37° C. The digest is then filtered, using Munktell's No. 8 filter paper, made up to 2500 cc. with water, and to it are added 10 gm. of dipotassium hydrogen phosphate and 5 gm. of magnesium sulphate. Sufficient 12 *N* sodium hydroxide is added to adjust the broth to a pH of 6.8. Note: this broth approximates that from which Orla-Jensen prepares, by dilution with an equal volume of water, the standard casein digest broth used by him in his fermentation studies (2).

2 Peptic casein digest broth, prepared by diluting No. 1 with an equal volume of water. Note: This broth approximates the standard casein digest broth of Orla-Jensen (2).

3 Peptic casein digest broth, prepared in the following manner: to 3000 cc. of tap water are added 140 gm. of commercial casein (Will Corp.), 8 gm. of pepsin and 36 cc. of concentrated hydrochloric acid. With frequent shaking the whole is allowed to digest for one day at 37° C.: subsequent preparation as described for No. 1.

4 Peptic casein digest broth: prepared as No. 3 except that digestion continues for two days.

5 Peptic casein digest broth: prepared as No. 3 except that digestion continues for 10 days.

6 Peptic casein digest broth: prepared as No. 1 but digestion continuing for one day only.

7 No. 6 diluted with an equal volume of water.

8 Peptic casein digest broth: prepared as No. 1 but digestion continued for two days only.

9 No. 8 diluted with an equal volume of water.

10 Peptic casein digest broth: prepared as No. 1.

11 No. 10 diluted with an equal volume of water.

12 Peptic casein digest broth prepared from Vitamine-Free casein (B.D.H.): method of preparation as described for No. 1.

13 No. 12 diluted with an equal volume of water.

14 Peptic casein digest broth prepared from acetic precipitated soluble casein (B.D.H.): method of preparation as for No. 1.

15 No. 14 diluted with an equal volume of water.

16 Peptic casein digest broth prepared from light white soluble casein (B.D.H.): method of preparation as for No. 1.

**After preparation, each respective broth, in quantities not greater than 1500 cc., is sterilized for 25 min. at 12 lb. pressure.*

17 No. 16 diluted with an equal volume of water.

18 Peptic casein digest broth prepared from casein made according to Plimmer (4): method of preparation as for No. 1.

19 No. 18 diluted with an equal volume of water.

20 Tryptic casein digest broth prepared as follows: to 500 cc. of 1% sodium carbonate solution is added 45 gm. of commercial casein (Will Corp.). The solution is allowed to remain at 37° C. for 24 hr., and 50 cc. of an 87% glycerol extract (10 parts 87% glycerol to 1 part dry gland) of dried pig's pancreas prepared according to the method of Willstätter (7) is then added. Digestion is continued for 10 days, the pH of the digest being adjusted to pH 7.6 from time to time. The digest is then filtered, made up to 420 cc. with water, and to it are added 1.67 gm. of dipotassium hydrogen phosphate and 0.88 gm. of magnesium sulphate. Sufficient 6 *N* hydrochloric acid is then added to adjust the broth to a pH of 6.8.

21 No. 20 diluted with an equal volume of water.

22 Peptic blood fibrin digest broth prepared as follows: to 375 cc. of tap water are added 35 gm. of blood fibrin*, 2 gm. of pepsin and 4.5 cc. of concentrated hydrochloric acid. With frequent shaking the whole is allowed to digest for six days at 35° C. On the second and third days respectively 0.5 cc. of concentrated hydrochloric acid is added to the digest. The digest is then filtered, made up to 325 cc. with water, and to it are added 1.25 gm. of dipotassium hydrogen phosphate and 0.63 gm. of magnesium sulphate. Sufficient 12 *N* sodium hydroxide is then added to adjust the broth to a pH of 6.8.

23 No. 22 diluted with an equal volume of water.

24 Peptic blood fibrin digest broth: prepared as No. 22 except that the digestion continues for 10 days.

25 No. 24 diluted with an equal volume of water.

26 Witte's peptone broth: 69.6 gm. of Witte's peptone, 4 gm. of dipotassium hydrogen phosphate and 2 gm. of magnesium sulphate dissolved in one litre of water.

27 No. 26 diluted with an equal volume of water.

28 Difco proteose peptone broth: 74.0 gm. of proteose peptone (Difco), 4 gm. of dipotassium hydrogen phosphate and 2 gm. of magnesium sulphate dissolved in one litre of water.

29 No. 28 diluted with an equal volume of water.

30 Hydrolyzed casein (Difco) broth—Bacto tryptophane broth—: 83.2 gm. hydrolyzed casein, 4 gm. of dipotassium hydrogen phosphate and 2 gm. of magnesium sulphate dissolved in one litre of water.

31 No. 30 diluted with an equal volume of water.

**Blood fibrin was prepared from fibrin as obtained from the abattoir by washing with water for 24 hr., grinding, again washing with water for 24 hr., and then dehydrating by successive treatment with 50, 75, 90 and 95% alcohol. It was then dried at room temperature and ground to a fine powder.*

32 Bacto-peptone broth: 65.2 gm. Bacto peptone, 4 gm. of dipotassium hydrogen phosphate and 2 gm. of magnesium sulphate dissolved in one litre of water.

33 No. 32 diluted with an equal volume of water.

34 Bacto yeast extract broth: 150.6 gm. of yeast extract (Bacto) added to approximately 700 cc. of water, heated to 80° C. and filtered. The residue on the filter paper is well washed with warm water, and filtrate and washings cooled and made up to 1000 cc. with water.

35 No. 34 diluted with an equal volume of water.

36 Marmite broth: 159.4 gm. of marmite made up to one litre with water: method of preparation as described for No. 34.

37 No. 36 diluted with an equal volume of water.

38 Yeast Vitamine-Harris broth: 1630 yeast Vitamine-Harris tablets made up to one litre with water: method of preparation as described for No. 34.

39 No. 38 diluted with an equal volume of water.

40 Yeast extract (from Orla-Jensen direct) broth: 161.0 gm. of yeast extract made up to one litre with water: method of preparation as described for No. 34.

41 No. 40 diluted with an equal volume of water.

42 Bacto peptonized milk broth: 156.9 gm. of peptonized milk (Bacto) dissolved in one litre of water.

43 Bacto-gelatin broth: 65.5 gm. of gelatin (Bacto) dissolved in one litre of water.

The results of the determinations of the nitrogen distribution in the sources defined and described are given in Table I.

The results of the analyses show that peptic casein digest broth prepared from commercial casein (Will Corporation), acetic precipitated soluble casein (B.D.H.) or light white soluble casein (B.D.H.) contains from 55 to 63% of proteose nitrogen, 19 to 25% of peptone nitrogen and 14 to 17% of subpeptone nitrogen according to the particular casein used, when the standard method of preparation is followed (2, 3). The distribution of the nitrogen in these three digests is of the same order. If less casein is used for digestion or if the period of digestion is reduced, the total amount of nitrogen made available is lower; this being true for the subpeptone nitrogen fraction in particular.

The peptic digest of blood fibrin contains equal amounts of the proteose nitrogen and peptone nitrogen fractions; and the subpeptone nitrogen fraction is comparable with this fraction of the peptic casein digests.

In the tryptic casein digest broth prepared from commercial casein, 70% of the nitrogen is in the subpeptone nitrogen fraction, and about 28% is found as peptone nitrogen.

Of the commercial sources analyzed, Difco proteose peptone broth and Bacto-peptone broth show a nitrogen distribution of the same order, and dissimilar from the distribution found in any other source analyzed. Both sources contain much more peptone nitrogen, more subpeptone nitrogen and

TABLE I

NITROGEN DISTRIBUTION IN DEFINED NITROGEN SOURCES

Source of nitrogen Number*	Nitrogen in grams per 100 cc. of broth					Per cent of total nitrogen			
	Total nitrogen	Protein nitrogen	Proteose nitrogen	Peptone nitrogen	Subpeptone nitrogen	Protein nitrogen	Proteose nitrogen	Peptone nitrogen	Subpeptone nitrogen
1	0.966	0.046	0.556	0.196	0.166	4.76	57.69	20.36	17.19
2	0.483	0.023	0.278	0.098	0.083	4.76	57.69	20.36	17.19
3	0.409	0.059	0.284	0.043	0.023	14.41	69.42	10.40	5.77
4	0.453	0.023	0.326	0.074	0.030	4.99	72.05	16.24	6.72
5	0.494	0.019	0.348	0.067	0.060	3.53	70.52	13.52	12.43
6	0.500	0.054	0.318	0.096	0.032	11.05	63.97	19.05	5.93
7	0.250	0.027	0.159	0.048	0.016	11.05	63.97	19.05	5.93
8	0.724	0.044	0.460	0.160	0.060	5.96	63.57	22.07	8.40
9	0.362	0.022	0.230	0.080	0.030	5.96	63.57	22.07	8.40
10	0.894	0.040	0.598	0.168	0.088	4.41	65.32	18.32	11.95
11	0.447	0.020	0.299	0.084	0.044	4.41	65.32	18.32	11.95
12	0.552	0.0012	0.300	0.154	0.086	0.22	54.28	27.75	17.75
13	0.275	0.0006	0.150	0.077	0.043	0.22	54.28	27.75	17.75
14	0.816	0.0172	0.514	0.160	0.124	2.10	63.04	19.66	15.20
15	0.408	0.0086	0.257	0.080	0.062	2.10	63.04	19.66	15.20
16	0.906	0.044	0.502	0.232	0.128	4.86	55.39	25.66	14.09
17	0.453	0.022	0.251	0.116	0.064	4.86	55.39	25.66	14.09
18	1.032	0.074	0.632	0.228	0.098	7.16	61.29	22.16	9.39
19	0.516	0.037	0.316	0.114	0.049	7.16	61.29	22.16	9.39
20	1.068	0.026	0.0	0.296	0.746	2.50	0.0	27.68	69.82
21	0.534	0.013	0.0	0.148	0.373	2.50	0.0	27.68	69.82
22	0.960	0.054	0.400	0.366	0.140	5.66	41.72	38.17	24.45
23	0.480	0.027	0.200	0.183	0.070	5.66	41.72	38.17	24.45
24	1.176	0.030	0.480	0.464	0.202	2.55	40.78	39.38	17.29
25	0.588	0.015	0.240	0.232	0.101	2.55	40.78	39.38	17.29
26	1.000	0.229	0.464	0.229	0.078	22.88	46.43	22.93	7.76
27	0.500	0.115	0.232	0.114	0.039	22.88	46.43	22.93	7.76
28	1.000	0.0	0.297	0.478	0.225	0.0	29.68	47.84	22.48
29	0.500	0.0	0.149	0.239	0.112	0.0	29.68	47.84	22.48
30	1.000	0.048	0.358	0.273	0.321	4.82	35.84	27.26	32.08
31	0.500	0.024	0.179	0.137	0.165	4.82	35.84	27.26	32.08
32	1.000	0.0	0.215	0.494	0.291	0.0	21.55	49.43	29.02
33	0.500	0.0	0.108	0.247	0.145	0.0	21.55	49.43	29.02
34	1.000	0.0	0.0	0.242	0.758	0.0	0.0	24.20	75.80
35	0.500	0.0	0.0	0.121	0.379	0.0	0.0	24.20	75.80
36	1.000	0.042	0.0	0.394	0.564	4.22	0.0	39.29	56.49
37	0.500	0.021	0.0	0.197	0.282	4.22	0.0	39.29	56.49
38	1.000	0.0	0.0	0.368	0.632	0.0	0.0	36.86	63.14
39	0.500	0.0	0.0	0.184	0.316	0.0	0.0	36.86	63.14
40	1.000	0.022	0.0	0.326	0.652	2.19	0.0	32.59	65.22
41	0.500	0.011	0.0	0.163	0.326	2.19	0.0	32.59	65.22
42	1.000	.0362	0.059	0.430	0.480	3.62	5.29	43.05	48.04
43	1.000	0.027	0.793	0.156	0.030	2.07	79.31	15.61	3.01

**See foregoing definitions and descriptions.*

much less proteose nitrogen respectively than the peptic digests of casein contain. In Witte's peptone the subpeptone nitrogen fraction is little greater than the subpeptone nitrogen fraction in a broth prepared by digesting commercial casein with pepsin for two days only.

The nitrogen distribution in yeast extract (Difco) broth is of the same order as the distribution in the standard tryptic casein digest.

Hydrolyzed casein (Difco) broth presents a nitrogen distribution picture that is something of a composite of the nitrogen distribution in the standard peptic casein digest and the tryptic casein digest.

When the nitrogen sources fractionated are employed as the substrate for fermentation studies, it will be seen that whilst the suitability of a source is not always fully indicated by the nitrogen distribution picture, the biological significance of the nitrogen distribution in the sources is, on the whole, reflected in the influence on the sugar-fermenting abilities of the lactic acid bacteria reported upon.

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CHEESE-RIPENING STUDIES[1]

Nitrogen Requirements of Lactic Acid Bacteria

II. THE INFLUENCE OF DEFINED NITROGEN SOURCES ON THE SUGAR-FERMENTING ABILITIES OF LACTIC ACID BACTERIA

By Wilfrid Sadler[2], Blythe Alfred Eagles[3] and Gladys Pendray[4]

Abstract

The influence of 36 nitrogen sources on the sugar-fermenting abilities of five cultures isolated from cheese has been studied. The five cultures are Gram positive coccus forms that fail to liquefy gelatin: some appear as chains in young milk culture and some are seen as pairs. Three sugars, glucose, mannose and lactose have been used for the work. The fermentation studies have been done after the manner of Orla-Jensen: the suitability of a nitrogen source being interpreted in terms of the total titratable acidity produced by the organisms from defined sugars, after 14 days incubation at the appropriate temperature.

For each organism, peptic casein digest broth is a very suitable source of nitrogen when the standard method of preparation is followed, and the total nitrogen content of the broth is approximately 1%. If the broth be diluted to contain 0.5% total nitrogen, the total titratable acidity obtainable is commonly less by one-third. Containing approximately 1% total nitrogen or 0.5% total nitrogen, tryptic casein digest broth is unsatisfactory as a nitrogen source for cultures EMB_1 173 and 195; but is very suitable for cultures EMB_2 166, 168 and 173,—providing the total nitrogen content of the broth is 0.5% rather than 1% total nitrogen.

In the broth prepared from two commercial peptones, the nitrogen distribution is of the same order, but in each case dissimilar from the nitrogen distribution in peptic casein digest or in tryptic casein digest: one peptone broth—1% total nitrogen content—is a very suitable source of nitrogen for all the organisms; but, apart from the fermentation of mannose by two strains, the other peptone broth is no more satisfactory than is a peptic casein digest containing 0.5% nitrogen. In a commercial hydrolyzed casein broth, the nitrogen distribution is something of a composite picture of the distribution in peptic casein digest broth and tryptic casein digest broth. This source is less suitable for culture EMB_1 173 than is peptic casein digest broth, equally suitable with peptic casein digest for culture EMB_1 195 and, for cultures EMB_2 166, 168 and 173, is the best nitrogen source investigated. The hydrolyzed casein broth containing 1% total nitrogen is much more suitable for each culture than is the same broth diluted to contain 0.5% total nitrogen.

Differentiation as between cultures EMB_1 173 and 195, on the one hand, and cultures EMB_2 166, 168 and 173, on the other hand, may be obtained by employing certain of the nitrogen sources investigated.

It has been shown that, when the nitrogen sources fractionated are employed as the substrate for fermentation studies, the suitability of a source is not necessarily indicated by the nitrogen distribution picture: even so, it is to be seen that, on the whole, the biological significance of the nitrogen distribution in a source is reflected in the influence on the sugar-fermenting abilities of the lactic acid bacteria reported upon.

The results of the fermentation study show clearly that if the "kind" of nitrogen made available is suitable, the "amount" of nitrogen supplied is then equally important.

For this study the authors chose five cultures on which they had been working for over a year. Cultures EMB_1 173 and 195 were isolated from a Kingston cheese of the "make" of December 4, 1929—at the time the cheese

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was two weeks old—and are two of some 365 strains then recovered. Cultures EMB_2 166, 168 and 173 were isolated from another Kingston cheese of the "make" of December 4, 1929, at the time the cheese was two months old. The five cultures are Gram positive coccus forms and fail to liquefy gelatin: some appear as chains in young milk culture and some are to be seen as pairs: the total titratable acidity produced by each strain in milk and in milk enriched with yeast extract has been determined repeatedly (6). The casein-splitting abilities of the organisms have been determined (2) and the work repeated some months later, and the effect on their sugar-fermenting abilities of enriching the nitrogen source with yeast extract has been studied (5).

Experimental

Employing each of 36 nitrogen sources (1), as the substrate, the authors prepared glucose, mannose and lactose broth respectively.

In each case the respective sugar is added at the rate of 2%. The broth is then tubed and plugged—10 cc. in each test tube—and is sterilized at 12 lb. pressure for 20 min. After sterilization each tube is inoculated with the desired culture. Uniformly a 2-mm. loop inoculation from a vigorous growth in milk or casein digest broth is made—milk or broth used for this purpose being enriched with a trace of yeast extract. After inoculation, the series with controls are incubated for 14 days at the appropriate temperature—in this case 23°C. When incubation is completed, the cultures are titrated with *N*/4 sodium hydroxide using phenolphthalein as indicator; the titration of the controls deducted and the results worked out and recorded as grams lactic acid per mille. It can be seen, that in the preparing of a sugar broth and in the manner of incubating, titrating, and recording of results the authors follow the procedure employed by Orla-Jensen (1, 3, 4).

The results of the determinations of the total titratable acidity produced by each of the five cultures are given in Table I.

Discussion

The total titratable acidity produced from each of the three sugars shows that when the total nitrogen content of the broth is approximately 1%, the peptic casein digest prepared from commercial casein (Will Corporation), source No. 1, is a very suitable source of nitrogen for all the cultures. When the same broth is diluted so that the total nitrogen content is approximately 0.5%, source No. 2, this source is not unsuitable in terms of "kind" of nitrogen; but the amount of acid produced by each organism from the respective sugars is reduced to the extent of about one-third, Table I (see also (1), Table I). The titration figures indicate that if the "kind" of nitrogen available is suitable, the "amount" of nitrogen supplied is equally important—interpreting the suitability of a nitrogen source in terms of the total titratable acidity produced by the organisms from the sugars submitted to fermentation.

The effect of departing from the usual method of preparing a peptic casein digest broth (1, 3, 4), may be seen in the nitrogen distribution in sources

Nitrogen source* Number	Culture EMB$_1$ 173			Culture EMB$_1$ 195			Culture EMB$_2$ 166			Culture EMB$_2$ 168			Culture EMB$_2$ 173		
	Dextrose	Mannose	Lactose	Dextrose	Mannose	Lactose	Dextrose	Mannose	Lactose	Dextrose	Mannose	Lactose	Dextrose	Mannose	Lactose
1	7.2	7.7	8.3	6.8	7.0	6.5	6.3	6.8	7.4	6.1	6.3	7.7	5.2	6.3	7.7
2	4.5	4.5	4.5	4.1	4.5	4.7	3.6	4.3	5.0	3.6	4.1	4.7	3.2	2.9	4.5
3	5.2	5.0	4.3	4.7	4.7	3.8	3.2	2.3	2.5	3.4	2.7	2.3	3.4	2.5	2.7
4	5.2	5.0	4.7	5.0	4.7	3.8	4.3	2.9	3.8	4.7	3.4	3.6	5.4	4.1	5.0
5	6.1	5.9	5.6	5.6	5.4	5.4	3.2	2.7	3.6	3.4	2.0	3.2	4.1	2.5	3.6
6	5.0	5.4	4.7	5.6	5.9	5.2	3.2	2.7	3.8	3.6	3.2	3.2	3.8	3.2	4.5
7	3.4	3.2	2.9	3.6	3.4	3.2	2.0	1.8	1.8	1.8	1.8	1.6	2.0	1.8	2.0
8	5.9	5.9	5.2	5.6	5.4	5.2	4.1	3.2	4.3	4.7	3.8	4.3	5.6	3.6	5.4
9	3.6	3.4	3.4	3.4	3.4	3.4	2.3	1.8	2.0	2.5	1.8	2.3	2.3	1.6	2.3
10	7.2	5.9	6.3	7.0	5.9	5.9	5.6	4.3	4.7	5.9	4.3	5.9	7.7	5.6	7.7
11	4.1	3.8	4.1	3.6	3.6	3.8	3.4	2.9	3.8	3.4	3.2	3.6	3.8	3.2	4.5
12	4.1	3.2	3.4	5.6	4.3	3.6	4.1	5.4	3.6	4.3	6.8	3.8	3.6	5.9	2.5
13	4.1	3.2	2.9	3.8	3.8	2.3	2.0	3.8	2.5	2.3	4.3	2.3	2.3	4.1	1.8
14	6.5	6.5	5.4	7.9	6.1	6.1	8.1	9.9	7.2	8.3	9.0	7.0	7.4	8.6	6.1
15	4.3	3.8	3.6	4.7	4.3	4.1	5.4	6.8	4.5	5.9	6.8	4.5	5.0	6.1	3.2
16	7.7	7.2	7.0	7.4	6.8	6.5	7.2	8.6	7.4	7.7	8.1	7.2	7.7	9.0	7.7
17	4.7	4.3	4.3	4.7	3.8	4.1	4.5	5.6	4.7	4.7	5.9	4.5	4.7	5.0	4.3
19	4.7	4.3	4.3	4.1	3.6	3.8	2.3	1.6	1.8	1.8	1.8	1.6	2.9	1.8	1.8
20	0.7	0.7	1.4	0.9	0.7	2.7				2.5	2.9	3.2	2.3	2.5	4.3
21	2.5	1.6	2.7	2.7	2.3	3.2	8.3	7.4	6.5	7.4	7.2	5.9	8.3	8.6	2.9
22	7.4			6.3			3.8			4.3			4.7		
23	4.7	4.3	2.5	4.3	3.4	3.6	3.2	3.2	2.7	2.7	2.9	2.5	6.1	3.8	3.4
24	9.5			8.1			4.3			4.1			7.2		
25	5.6	5.0	5.0	5.2	5.0	4.3	2.7	2.3	2.5	2.0	2.5	2.0	4.5	4.1	3.2
26	6.8	6.3	6.3	7.0	7.0	6.3	2.3	2.7	3.8	2.7	1.1	3.8	1.8	1.6	3.8
27	3.8	3.4	3.6	3.8	4.7	3.8	1.6	1.6	2.0	1.8	2.0	2.0	1.1	1.4	2.0
28	8.1	7.7	5.9	8.1	7.2	6.3	6.5	7.4	6.3	6.8	8.1	6.1	7.9	9.0	7.4
29	5.2	4.7	3.8	5.0	4.5	3.8	4.5	6.1	4.3	5.0	5.9	4.7	6.3	7.7	5.9
30	6.5	6.3	6.5	7.0	7.4	7.2	10.4	10.6	11.5	9.0	10.1	11.5	11.0	11.7	12.2
31	4.5	4.5	4.3	4.3	4.3	4.1	7.7	8.3	8.1	7.4	8.1	8.1	7.4	9.0	8.8
32	5.6	4.3	4.3	4.5	4.1	4.5	3.6	6.5	3.6	4.1	6.8	4.7	3.8	2.9	5.0
33	3.2	2.9	2.7	2.9	2.9	2.5	1.8	5.4	2.0	2.0	5.6	2.5	2.3	6.1	2.9
34	0.0	0.0	0.0	2.5	1.4	1.8	5.2	6.5	3.4	5.4	7.0	4.1	6.5	8.1	4.3
35	2.7	1.6	2.0	3.6	2.9	2.0	3.8	6.3	3.8	3.6	6.1	3.6	4.7	6.8	4.1
36	0.7	0.0	0.5	0.9	1.1	1.8	0.5	0.7	0.7	0.5	1.1	0.9	0.9	0.9	1.6
37	1.8	1.1	0.9	1.8	2.3	1.1	2.3	2.7	2.3	2.7	2.9	2.3	2.7	5.4	

NOTE:—Results recorded as grams lactic acid per mille.

**For descriptions and analyses of nitrogen sources see paper by Eagles and Sadler (1).*

Nos. 3, 4, 5, 6 and 8 (1); and in the influence of the sources on the sugar-fermenting abilities of the organisms, Table I. It is to be seen that whilst the subpeptone nitrogen fraction in source No. 5 is low, this fraction is very low in sources Nos. 3, 4, 6 and 8 (1); and there would appear to be a relation of the order of the nitrogen distribution to the low titratable acidity produced by cultures EMB_2 166, 168 and 173 from each of the three sugars, Table I. Even for cultures EMB_1 173 and 195 these digests cannot compare as nitrogen sources with the casein digest broth supplied as sources Nos. 1, 14 or 16, Table I.

According to the method of preparation, the peptic casein digest broth source No. 10 should be closely comparable with source No. 1 (1). The nitrogen breakdown in the former however is not as complete as in the latter: much less subpeptone nitrogen is made available (1), and the source is less suitable for cultures EMB_2 166 and 168, Table I. On the other hand, for the fermentation of dextrose by cultures EMB_1 173 and 195, and the fermentation of dextrose and lactose by culture EMB_2 173, the nitrogen supplied as source No. 2 is satisfactory, Table I. By comparing the influence of nitrogen source No. 10 and the diluted broth No. 11 on the amount of acid produced by the organisms, the importance of "amount" as well as "kind" of nitrogen supplied is clearly shown, Table I.

Both the peptic casein digest broth prepared from acetic precipitated soluble casein (B.D.H.) and light white soluble casein (B.D.H.), sources Nos. 14 and 16 respectively (1), are very good sources of nitrogen for the five organisms; and for cultures EMB_2 166, 168 and 173 in particular, Table I. For the fermentation of dextrose and mannose by these three strains there appears to be a specific value in the peptic digests of the B.D.H. caseins—a value that is not apparent in the digest of Will Corporation commercial casein, source No. 1, Table I. As was shown for the peptic casein digest broth prepared from Will Corporation commercial casein, Table I, so with the peptic casein digest broth prepared from acetic precipitated soluble casein (B.D.H.) and light white soluble casein (B.D.H.) respectively—the broth containing the greater total nitrogen content (1) being the more suitable source of nitrogen for the fermentation studies on the five cultures, Table I. Both the "kind" and "amount" of nitrogen supplied are seen to be important.

On digesting Vitamin-Free casein (B.D.H.) with pepsin, it has been seen that the yield of available nitrogen is little more than half that obtained by the peptic digesting of light white soluble casein (B.D.H.) (1). We find however that the nitrogen distribution of the two is somewhat of the same order (1); and that the peptic digest broth prepared from Vitamin-Free casein (B.D.H.), source No. 12, is comparable in value as a nitrogen source for the five cultures, with the diluted peptic digest broth prepared from light white soluble casein (B.D.H.), source No. 17, Table I, (see also (1) Table I). These comparisons are of peculiar interest.

The peptic casein digest broth, source No. 18, prepared from casein (Plimmer) provides a comparatively small amount of the subpeptone nitrogen fraction (1). The digestion continued for 10 days (1); yet it has been seen that

the nitrogen distribution is almost identical with that of source No. 8, a peptic casein digest broth prepared from commercial casein (Will Corporation) by digesting for two days only (1). When employing the diluted broth of each, Nos. 19 and 9, as nitrogen sources for the fermentation studies, the total titratable acidity produced by cultures EMB_2 166, 168 and 173 respectively is low and almost identical in amount, Table I: low total titratable acidity when supplied with a nitrogen source poor in the subpeptone nitrogen fraction. As a nitrogen source for cultures EMB_1 173 and 195, source No. 19 is comparable in value with source No. 2, the diluted peptic digest broth prepared from commercial casein (Will Corporation), Table I.

If now we compare No. 19 with No. 2—the diluted peptic digest of commercial casein (Will Corporation)—each broth being the result of a 10-day period of digestion (1), the former is seen to provide little more than half the amount of subpeptone nitrogen yielded by the latter; the differences being small in the peptone nitrogen and proteose nitrogen fractions respectively (1). The differences in the nitrogen distribution are reflected in the influence on the sugar-fermenting abilities of three of the organisms. Supplied with No. 19 as the nitrogen source, cultures EMB_2 166, 168 and 173 produce less total titratable acidity than when supplied with No. 2, Table I: the lower amount of acid being produced when the nitrogen source containing the lesser subpeptone nitrogen fraction is supplied. No such significance is apparent in the case of cultures EMB_1 173 and 195, Table I.

Although the peptic digest of blood fibrin, source No. 24, contains less proteose nitrogen and much more peptone nitrogen than, say, the peptic casein digest source No. 1 (1), the former is much less suitable as a nitrogen source for cultures EMB_2 166 and 168 than is the latter, Table I—the provision of an adequate supply of the subpeptone nitrogen fraction and the peptone nitrogen fraction notwithstanding. On the other hand, blood fibrin digest is slightly superior to the casein digest as a nitrogen source for cultures EMB_1 173 and 195 and culture EMB_2 173, Table I. Of necessity, the diluted broth of each source has been considered in these comparisons. The subpeptone nitrogen and peptone nitrogen fractions in the peptic blood fibrin digest apparently fail to influence the sugar-fermenting abilities of cultures EMB_2 166 and 168 to the same extent as do these fractions when contained in a peptic casein digest, Table I (see also (1) Table I). Hence it may be that suitability as a nitrogen source for fermentation studies is not fully indicated by the nitrogen distribution picture of the source.

It has been seen that the tryptic casein digest broth, source No. 20, is made up of 2.50% protein nitrogen, 27.68% peptone nitrogen and 69.82% subpeptone nitrogen; and that there is an entire absence of the proteose nitrogen fraction (1). The broth containing approximately 1% nitrogen is unsuitable as a nitrogen source for any of the five cultures, Table I. The diluted broth, total nitrogen content approximately 0.5%, source No. 21, is also quite unsuitable as a nitrogen source for cultures EMB_1 173 and 195, Table I. On the other hand, when the dilute broth is employed, the total titratable acidity produced by cultures EMB_2 166, 168 and 173 is high, Table I—interestingly

enough, the figures being comparable with those obtained when the nitrogen source is the peptic digest broth (0.908% total nitrogen) prepared from light white soluble casein (B.D.H.), Table I: and the difference in the nitrogen distribution in the two sources is marked. Again there are clear indications that both the "kind" and "amount" of nitrogen supplied are critical. In the two concentrations, the kind of nitrogen available in the tryptic casein digest is unsuitable for cultures EMB_1 173 and 195. In the higher concentration, tryptic casein digest broth is unsuitable for cultures EMB_2 166, 168 and 173; yet when the diluted broth is used, not only the "kind" but also the "amount" of nitrogen contained is particularly suitable as a nitrogen source for the fermentation of the sugars by these cultures, Table I.

Witte's peptone broth, source No. 26, is low in the subpeptone nitrogen fraction and contains a higher percentage of protein nitrogen than does any other source analyzed (1). Provided the total nitrogen content of the broth is 1%, Witte's peptone is suitable for cultures EMB_1 173 and 195, but unsuitable for cultures EMB_2 166, 168 and 173; diluted to contain 0.5% total nitrogen, source No. 27, Witte's peptone broth is inadequate as a nitrogen source for any of the five cultures, Table I.

It may be seen (1) that the nitrogen distribution in proteose peptone (Difco) broth, source No. 28, and Bacto peptone broth, source No. 32, is somewhat of the same order, and is dissimilar from the distribution found in any other source analyzed. Also it is seen (1) that both sources contain much more peptone nitrogen, more subpeptone nitrogen and much less proteose nitrogen than the peptic digests of casein contain (1). Except for the fermentation of mannose by cultures EMB_2 166 and 168, Bacto peptone broth—or the same broth diluted, source 33—is inadequate as a source of nitrogen for determining the sugar-fermenting abilities of any of the five cultures, Table I. On the other hand, proteose peptone (Difco) broth, 1% total nitrogen content, is a good nitrogen source for each of the five cultures and, nitrogen content for nitrogen content, compares favorably with the peptic casein digests, Table I. In common with the peptic casein digest prepared from light white soluble casein (B.D.H.), source No. 16, proteose peptone (Difco) broth appears to have a specific value as a nitrogen source for the fermentation of mannose by cultures EMB_2 166, 168 and 173, Table I (see also (1)).

From Table I, and (1) it is seen that, showing a nitrogen distribution picture somewhat of the same order, proteose peptone (Difco) broth and Bacto peptone broth are not at all comparable, the one with the other, when the value of each as a nitrogen source is reflected in the sugar-fermenting abilities of the five cultures, Table I. Again there is the suggestion that the nitrogen distribution in a source does not necessarily give a full indication as to the suitability or otherwise of the source for the determining of the sugar-fermenting abilities of these cultures (1), Table I.

In Bacto yeast extract broth, source No. 34, the nitrogen content consists entirely of subpeptone nitrogen 75.80% and peptone nitrogen 24.20%, the subpeptone nitrogen fraction being a little greater and the peptone nitrogen fraction a little less than these fractions respectively in tryptic casein digest,

source No. 20 (1). As has been seen (1), apart from a small percentage of protein nitrogen in tryptic casein digest, the two sources are almost identical in their nitrogen distribution. This marked similarity is reflected in the influence on the sugar-fermenting abilities of cultures EMB_1 173 and 195. The yeast extract broth, as the tryptic casein digest broth, is unsuitable as a nitrogen source for the fermentation of the three sugars by these two cultures—whether the original broth (1% total nitrogen content) or the diluted broth (0.5% total nitrogen content) source No. 35, be employed, Table I. For cultures EMB_2 168 and 173, yeast extract broth having a total nitrogen content of 1% is much more suitable as a nitrogen source than is tryptic casein digest broth containing approximately 1% nitrogen, but much less suitable when the diluted broth is used, Table I. Yeast extract broth does not compare favorably with peptic casein digest broth or with hydrolyzed casein (Difco) broth as a nitrogen source for the fermentation of glucose and lactose by cultures EMB_2 166, 168 and 173, but there is a specificity in yeast extract as a nitrogen source for the fermentation of mannose by these three strains, Table I (see also (1) Table I)—a specificity comparable with that observed when discussing Bacto peptone broth and the peptic digests of casein (B.D.H.).

The unsuitability of marmite as a nitrogen source for each of the five cultures is a curious finding, Table I, particularly when the nitrogen distribution picture is compared with the nitrogen distribution in yeast extract broth and in tryptic casein digest broth respectively (1). The authors can offer no comment.

In its nitrogen distribution, hydrolyzed casein (Difco) broth presents something of a composite picture of the nitrogen distribution in a peptic casein digest and a tryptic casein digest (1), and when employed as a nitrogen source reflects this composite quality in the influence on the total titratable acidity produced from each of the sugars by each of the five cultures, Table I. Hydrolyzed casein broth is found to contain less proteose nitrogen, more peptone nitrogen and much more subpeptone nitrogen than the peptic casein digests Nos. 1, 14 or 16 contain; and, as compared with the tryptic casein digest, less than half the amount of subpeptone nitrogen and 35% more proteose nitrogen (1). Employed as a nitrogen source for cultures EMB_1 173 and 195, hydrolyzed casein broth is comparable with the peptic casein digests, sources No. 1, 14 or 16, Table I. For cultures EMB_2 166, 168 and 173, hydrolyzed casein broth is the most suitable source of nitrogen we have investigated, Table I. The influence as a nitrogen source is greater when the broth containing 1% total nitrogen rather than 0.5% total nitrogen is used, Table I. The pronounced suitability of hydrolyzed casein broth as a nitrogen source for the fermentation of the three sugars by cultures EMB_2 166, 168 and 173 is made quite clear by the data in Table I: the diluted broth, source No. 31, is approximately as suitable a nitrogen source as are the peptic digests of casein containing 1% total nitrogen; and as suitable as the tryptic casein digest broth containing approximately 0.5% total nitrogen, Table I. Again it is made clear that both the "kind" and the "amount" of nitrogen supplied are important.

Of itself, hydrolyzed casein broth, employed as a nitrogen source for the fermentation of the three sugars used, differentiates as between cultures EMB_1 173 and 195 on the one hand and cultures EMB_2 166, 168 and 173 on the other hand, Table I.

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NOTE ON AN INVESTIGATION INTO THE FLUORESCENCE OF HAIRS INFECTED BY CERTAIN FUNGI[1]

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Abstract

1. Observations under filtered ultra-violet light of the available material of *Microsporon audouini*, *M. felineum*, *Trichophyton gypseum*, *T. violaceum*, *T. album* and *Achorion schoenleini* indicate that the species belonging to the genera *Microsporon* and *Achorion* produce an intense green fluorescence in the substance of hairs infected by them. *Trichophyton*-infected hairs appear a paler bluish-white. Normal pigmented hairs appear dark.

2. It is shown that the spores and hyphae of the fungus whether within or without the hair are relatively non-fluorescent as contrasted with the substance of the infected hair.

3. A fluorescent substance present in hairs infected by *M. audouini*, *M. felineum* and *Achorion schoenleini* is readily soluble in hot water. No such substance could be extracted from the hairs infected by *Trichophyton sp.*, or from normal hairs. It is therefore believed that the presence or absence of the green fluorescence is pathognomonic.

Introduction

In 1925 Margarot and Devèze (3) observed the appearance of a green luminosity of the hairs from cases of favus and microsporosis examined in a darkened room by means of the ultra-violet radiation passing a filter of Wood's glass. The phenomenon was subsequently investigated by Vigne (6), Kinnear (2) and others, and the relevant literature up to 1929 is discussed in a later paper by Margarot and Devèze (4).

That the phenomenon has been utilized in the diagnosis of cases of ringworm of the scalp is shown by the numerous clinical references which have appeared during the last few years. The source of light usually employed is a mercury-arc, or carbon-arc lamp, fitted with Wood's glass or some other filter which has the property of absorbing most of the visible light and transmitting the long-wave ultra-violet radiation lying near to the visible region of the spectrum. A convenient portable lamp for this purpose has been developed by the present writers and described in another communication (1). If the beam of filtered ultra-violet light is allowed to fall upon the head of a child suffering from tinea capitis caused by *Microsporon audouini*, the affected areas are observed to shine with an intense emerald-green color, contrasting strongly with the normal parts of the scalp where the hairs usually appear dark or reflect some of the dim violet light which is not completely absorbed by the filter.

The present investigation is being carried out in order to determine whether or not fluorescence affords an accurate and rapid aid to diagnosis and a precise criterion of cure, as a preliminary step in a study of methods of

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treatment of ringworm of the scalp. Particular attention is being paid therefore to the limitations of the phenomenon in diagnosis, as a knowledge of these is necessary if valid conclusions are to be drawn from the test. The problem is being investigated in order to discover which species of fungi do, and which do not, cause the infected hairs to fluoresce, to determine how far infection is co-extensive with fluorescence, and to throw some light on the nature of the phenomenon.

The Fluorescence

Fluorescent hairs were studied in the laboratory by means of a microscope illuminated with a Leitz "Luminescence Lamp". This instrument consists of a carbon-arc lamp fitted with suitable filters which transmit ultra-violet light, but absorb visible radiations except those at the extreme violet and red ends of the spectrum. Another filter fitting over the eyepiece of the microscope is a complementary absorbent to the lamp filters, except in the extreme red region of the spectrum. Thus, on looking down the microscope tube, the empty field of view appears dark except for a red tinge. As the lamp and eyepiece filters are complementary absorbents, luminosity of an object placed in the field of the microscope must be due to fluorescence. As has been noted by previous writers, normal hairs examined by filtered ultra-violet light show a bluish-white fluorescence of various intensities, or appear dark, according to the absence or presence of pigment in the hair. A similar bluish-white fluorescence is noted in hairs infected by *Trichophyton*. Hairs infected by *Microsporon audouini*, or *M. felineum* fluoresce with a brilliant green color. By means of a Hilger Constant Deviation Spectroscope, preliminary examinations have been made of the fluorescent light emitted by uninfected white hairs, and by hairs attacked by *Achorion schoenleini*. Observations were difficult because individual readings differed considerably according to the degree to which the eye of the observer was accommodated to the very faint spectrum. A further inaccuracy was caused by the obvious differences in intensities of the fluorescent emissions from normal and infected hairs. The radiation from both sources consisted of a faint wide band extending from the yellow into the violet. At the yellow end, both bands terminated fairly sharply in the same region. The band spectrum of the uninfected hair, however, passed slightly farther into the violet than did that of the diseased hair. It remains to be shown by further studies whether this difference is enough to account for the difference in color between the hairs as perceived by the eye, or whether the colors may be in part an example of Purkinge's phenomenon caused by differences in the intensities of the emitted light. Evidence will be brought forward later to show that there is a difference in nature, if not necessarily in hue, between the fluorescence of normal, of *Microsporon*-infected, and of *Trichophyton*-infected hairs.

The phenomenon appears to fulfil two of the conditions which characterize fluorescence. (a) The light emitted by the luminescent hairs (green), to judge by the apparent hue, is of longer wave-length than the light exciting the emission (violet or ultra-violet). This conclusion is borne out by the fact that the

luminescence is not extinguished by the use of complementary absorbents. In the absence of direct spectroscopic measurements, however, the term "fluorescence" is applied to the phenomenon rather for convenience than as a result of strict proof. (b) The emission of the light appears to cease directly the exciting radiation is removed.

Relation between Fluorescence and Species of Fungus

Isolations of fungi have been made from hairs obtained from cases of ringworm of the scalp, beard, and trunk, and the organisms determined in culture upon Sabouraud's maltose "Proof" medium. The results of this study as far as they go are quite definite, and show that the species of fungus involved is the chief factor determining the presence or absence of fluorescence in the infected hair. Table I shows the correlation between the species of fungi isolated from ringworm-infected hairs, and the presence or absence of the emerald-green fluorescence in ultra-violet light.

TABLE I

CORRELATION BETWEEN SPECIES OF FUNGI ISOLATED FROM INFECTED HAIRS AND EMERALD-GREEN FLUORESCENCE UNDER ULTRA-VIOLET LIGHT

Organism	No. of cases from which isolated	No. of cases showing green fluorescence
Microsporon audouini	38	37*
Microsporon felineum	17	17
Trichophyton album	3	0
Trichophyton gypseum	2	0
Trichophyton violaceum	4	0
Achorion schoenleini	4	4

*+1 *Doubtful.*

In addition to the above cases from which the fungi have been isolated and identified in culture, there are a number of cases in which the organisms were classified as *Trichophyta* or *Microspora* on clinical and microscopical grounds, but were not grown and determined in culture. Table II shows the correlation between genus of parasite and the production of the green fluorescence in these cases where the study has been less thorough.

TABLE II

CORRELATION BETWEEN GENUS OF PARASITIC FUNGUS AND PRODUCTION OF FLUORESCENCE

Organism	No. of cases observed	No. of cases showing green fluorescence
Microsporon species	23	23
Trichophyton species	10	0

As indicated above, hairs invaded by *Trichophyton* species frequently show a bluish-white fluorescence of lower intensity than the green fluorescence of hairs infected by *Microsporon*.

These observations on the occurrence of the fluorescence show that: (a) the presence or absence of the green fluorescence in infected hairs is usually determined by the species of fungus infecting the hair; (b) infection by *Microsporon audouini*, *M. felineum*, or *Achorion schoenleini*

results in a strong green fluorescence; (c) infection by the species of *Trichophyton* studied causes a fluorescence resembling that of the normal stratum corneum.

Other Factors Conditioning Fluorescence

Although Tables I and II indicate that the species of parasite is largely responsible for the presence or absence of the green fluorescence, one doubtful case is recorded. This patient suffered from ringworm of the scalp caused by *Microsporon audouini* and was in contact with a number of other children similarly infected, all of whom showed the typical bright green fluorescence associated with *M. audouini*. When first examined, no trace of fluorescence could be observed over the areas in which typical broken hair-stumps could be found by means of a hand-lens. About 14 days later, the infected hairs showed a feeble green fluorescence when examined by the light of a water-cooled mercury-arc lamp fitted with a Wood's filter. Two weeks later the fluorescence had entirely disappeared. No satisfactory explanation for this variability in fluorescence is known to us.

In cases of infection by *Microsporon audouini* which were kept under observation over several months, variations were noted from time to time in the relative intensity of the green fluorescence manifested by different patients.

These observations are the only facts yet encountered which suggest that differences in the individual patient may sometimes determine the presence or absence of the fluorescence.

The Location of the Fluorescent Substance

An explanation of the fluorescence has been given by Margarot and Devèze (4). "La fluorescence propre des divers agents des teignes explique la teinte verdâtre observée en pareil cas. Les recherches de Vigne et les nôtres établissent que la luminosité est due au champignon lui-même. Nous avons pu nous rendre compte que dans le monde végétal d'autres champignons microscopiques étaient fluorescents." From this conclusion follows the view that the apparent non-fluorescence of the endothrix *Trichophyta* is due to the suppression of the luminosity by the matter surrounding the fungal hyphae distributed within the hair. Thus Vigne (6) wrote, "La fluorescence des cheveux teigneux étant due à la présence de spores, il n'est pas étonnant de constater que les cheveux contaminés par le Trichophyton, parasite endothrix, sont moins lumineux que ceux contaminés par le Microsporon dont les spores sont en très grand nombre et situées tout autour du cheveu." These explanations are regarded by the present writers as inadequate for the following reasons.

(1) In *Trichophyton* the ectothrix species *T. gypseum* and *T. album*, as well as the endothrix *T. violaceum*, do not exhibit the green fluorescence.

(2) Hairs infected by *Achorion schoenleini* in which the hyphae are situated almost entirely within the substance of the hair show the green fluorescence in a marked degree.

(3) The green fluorescence is not observed macroscopically in cultures of the organisms when grown on Sabouraud's medium. The cultures frequently

appear a violet color under the Wood's light, and a beautiful golden brown fluorescence has been noted from a culture of *Microsporon felineum*.

Moreover, for the following reasons the luminosity is believed to lie primarily within the substance of the hair.

(1) Microtome sections of hairs attacked by *Microsporon audouini* have been cut in the region of the follicle and spore sheath. By ultra-violet light with complementary filters, the whole section was visible, the follicle and spore-sheath appearing faintly bluish-white and the hair shaft bright green.

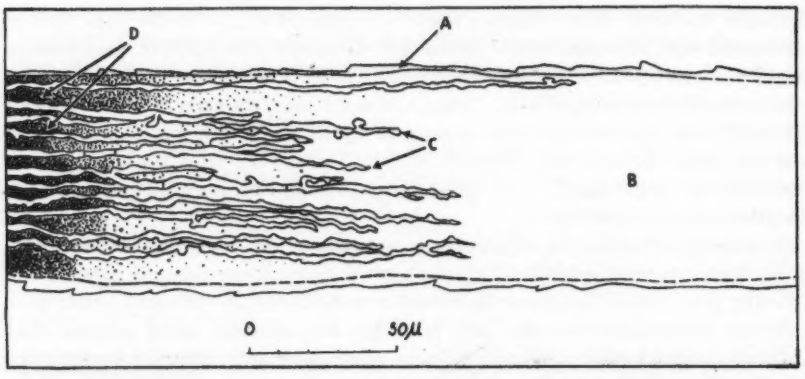

FIG. 1. *Semi-diagrammatic camera lucida drawing of region of Adamson's fringe in a hair infected by Achorion schoenleini, showing the relation between the hyphae of the fringe and the blue-green fluorescence (represented by shading) excited in the infected portion of the hair by the rays passing a filter of Wood's glass. A, cuticle of hair; B, uninfected portion of hair shaft between the bulb of the hair and infected region; C, the tips of the growing hyphae invisible by the Wood's light; D, the fluorescent part of the hair represented in reverse by shading, first appearing some little distance behind the edge of the fringe.*

(2) Microtome sections have been cut through the distal ends of hairs infected by *Achorion schoenleini*. Here the hyphae have mostly disintegrated, leaving channels in the keratin filled with air or with fat droplets. When examined microscopically with filtered ultra-violet light the cuticle and substance of the hair were seen to be fluorescent.

(3) Hairs infected by *Achorion schoenleini* were epilated intact and examined microscopically by filtered ultra-violet light in the region known as "Adamson's Fringe", where the invading fungus terminates as a fringe of hyphae just above the bulb of the hair. The fringe is thus the region of active invasion of the hair. The hyphae forming the fringe were invisible by ultra-violet light. The luminous portion of the hair "faded in" gradually at a short distance behind the fringe which is the region of active invasion. As illustrated semi-diagrammatically in Fig. 1, the hyphae are non-luminescent but the hair becomes luminous usually some little time after it is actually penetrated by the hyphae.

(4) Evidence was also obtained from a study of hairs newly infected by *Microsporon felineum*. It has been shown by Sabouraud (5) that before a hair is actually penetrated by the hyphae of *Microsporon*, broad ribbon-like hyphae

descend into the horny layers of the follicle. Some distance below the mouth of the follicle, the hyphae send branches inwards which enter the hair. Subsequently the mycelium grows downwards in the shaft of the hair, branching in the direction of the root. The part of the hair-shaft penetrated by the hyphae is covered by a sheath of spores developed from the infra-cuticular mycelium (Fig. 2, *A*). Such newly infected hairs were epilated together with the greater part of the follicle but not with the root. These were examined microscopically with filtered ultra-violet light. It could then be seen that only that part of the hair actually penetrated by the fungal hyphae was fluorescent (Fig. 3). The external mycelium surrounding the hair-shaft in the upper part of the follicle did not itself fluoresce or induce fluorescence in the hair substance beneath it (Fig. 2, *B*). The spore sheath (Fig. 2, *A*) appeared but feebly fluorescent (Fig. 3, *A*). The lower part of the hair where the spore-sheath had fallen off in epilating the hair showed that the hair-fibres were intensely fluorescent (Fig. 2, *C* and Fig. 3, *C*).

(5) The hyphae running longitudinally through hairs infected by *Achorion schoenleini* and *M. audouini* when examined microscopically by filtered ultra-violet light usually appear duller, but never, so far as observed, brighter than the adjacent part of the hair.

The conclusion drawn from the observations cited above is that the fluorescence is due to some change in the hair substance following invasion by the fungus. It is possible that an enzyme is excreted by the hyphae into the surrounding medium, and that some product of hydrolysis of the keratin or other body present in the hair may be the fluorescent substance.

The Fluorescent Substance

From *Achorion schoenleini*, *Microsporon audouini* and *M. felineum* a fluorescent substance has been extracted and obtained in the form of a fluorescent aqueous solution. Repeated attempts to extract a similar substance responsible for the pale blue or white fluorescence exhibited by hairs infected by *Trichophyton* species, or by normal hair, have been unsuccessful.

An experiment which has been repeated many times may be cited. From a scalp infected with *Achorion schoenleini* a small quantity of hair, about 0.2 mgm., was clipped from an infected area. A like sample was taken from an adjacent area of normal hair on the same scalp. When examined by means of the filtered ultra-violet light, the infected hairs were found to fluoresce while the normal hairs appeared dark. Any fluorescent hairs that had been gathered with the normal control sample could be removed at this stage. The samples were covered with ether and allowed to stand overnight. This treatment removed the natural fat of the hair. From the infected sample the fatty substance filling the channels in the hair, no doubt a product of fungal metabolism, was removed also. The removal of the fat allowed of wetting the hair thoroughly in the subsequent extraction. The ether was poured off, and the ethereal extracts from both the normal and the infected hairs could be seen to be non-fluorescent though they appeared faintly opalescent and

reflected some of the light which passed the filters. The appearance of the hairs by filtered ultra-violet light remained unchanged.*

The ether extracts containing the fat were evaporated down and clustered stellate fat-crystals soon appeared. The fat from both samples failed to show the green fluorescence. The dried samples of hair were then placed in test tubes, covered with about 10 cc. of distilled water, and heated in a water bath for a period of about an hour; the liquid was then filtered off and a further portion of water added to complete the extraction. The tubes were examined from time to time by the ultra-violet light, to check the progress of the extraction. When the filtered extracts from the two samples were thus examined, that from the infected hair was seen to fluoresce with a bright green color, while that from the normal hair was colorless. The nature of the fluorescent substance is under investigation.†

TABLE III

COMPARISON OF MICROSCOPICAL APPEARANCE OF HAIRS INFECTED BY *M. audouini* AND *T. gypseum*, WHEN SUBJECTED TO VARIOUS TREATMENTS SUCCESSIVELY

Examined dry with luminescence lamp	Water run under cover-slip	Boiled 1—1.5 min.	Dried in ether, examined again
Microsporon audouini			
Intense green fluorescence of hair. Spore sheath apparently transparent	No change	Fluorescent color diffused out into the water, specimen left very dull-looking	No change
Trichophyton gypseum			
Bluish-white fluorescence pronounced in corneous layers of follicle. Hair dark except at infected end which was somewhat blue-fluorescent, but less so than follicle.	Fluorescence of follicle dimmed at once. Hair became transparent, still appeared dark, except at infected end where feeble fluorescence was unchanged.	No change	Bluish-white fluorescence of follicle and infected portion of hair returned

**Incidentally when the hairs were allowed to dry after removing the ether, the infected hairs stood out prominently by reason of their lustreless grey color. This probably explains the well-known clinical test in which the scalp is washed with chloroform and the infected hairs are then seen to be frosted-white in appearance. The chloroform no doubt extracts the fatty substance present in the empty tubes left in the hair substance after the death of the fungus. When the fat solvent evaporates, the tubes become filled with columns of air. The greatly increased surface of hair-substance in contact with air reflects more light.*

†A preliminary chemical examination of the fluorescent extract has been made by Peter G. Mar, M.Sc. The aqueous extract obtained by the above procedure was concentrated by distillation at 35°—40° C. under reduced pressure. The concentrate (ca. 1 cc.), a light yellowish clear solution, still fluorescent under the filtered ultra-violet radiation, was then placed in a vacuum desiccator and dried over concentrated sulphuric acid. A small quantity of a somewhat hygroscopic substance remained, white, amorphous, powdery, and having a sharp musty odor.

Preliminary chemical tests with a small amount of the fluorescent solution indicated the presence of a neutral organic substance, or substances, containing nitrogen, an aldehyde group and a phenolic ring. Tests for carbohydrate, loosely combined sulphur and α-amino acids were negative. However, these results are put forward only provisionally, as the quantities used were too small to allow of purification and identification.

FIG. 2. *Hair and follicle without hair-bulb in early stage of infection by Microsporon felineum, photographed by visible light. A, the spore sheath surrounding the part of the hair penetrated by the fungus; B, the uninvaded portion of the hair shaft, outside which, however, the fungal hyphae could be seen on staining. C, the broken end of the hair.*

FIG. 3. *The same specimen photographed by ultra-violet light. A, faint fluorescence of the spore sheath; C, the broken end of the hair showing fluorescent hair fibres. The intensity of the fluorescence of the infected part of the hair fades rapidly towards the uninfected portion.*

Contrary to the experience of Kinnear (2), who found that the infected hairs retain their fluorescence in liquor potassae, it has been found that the extraction of the fluorescent substance may readily be demonstrated microscopically for single hairs by mounting them dry on a slide and running a drop of 7% potassium hydroxide under the cover-slip. The hairs are examined by ultra-violet light. Almost at once the fluorescence in the hair begins to fade, while the liquid in which the hair is mounted takes up the fluorescence.

Repeated comparisons have been made of the microscopical appearance of green-fluorescent hairs infected by *M. audouini* and the dull, bluish-white fluorescence of hairs infected by *T. gypseum*, when treated with boiling water. Typical observations are shown in Table III.

From these experiments it is concluded that a fluorescent substance can be extracted by warm water from hairs infected by *Achorion schoenleini*, by *Microsporon felineum* and by *Microsporon audouini*, but not from hairs infected by *Trichophyton gypseum*. The bluish-white fluorescence of these *Trichophyton*-infected hairs cannot therefore be due to the presence in smaller amount of the same substance as that producing the intense green fluorescence in hairs infected by *Achorion* and *Microsporon*. The difference observed between hairs infected by *Microsporon sp.* or *Achorion sp.* and hairs infected by *Trichophyton sp.* when examined by Wood's light is therefore considered to be a difference in kind and not merely of degree.

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IS THE DOOR OF *UTRICULARIA* AN IRRITABLE MECHANISM?[1]

By Francis E. Lloyd[2]

Abstract

The present paper deals with the question whether the door mechanism in *Utricularia* is an irritable one (as *e.g.*, Mimosa is irritable) or is mechanical, and in the opening paragraphs the two views are set forth together with mention of the protagonists and their factual contributions. In particular the recently published views of Kruck have been examined and found to be inadequate.

(a) The evidence for the conclusions herein presented have been drawn from a study of the posture of the door, and its exact positional relations to the threshold. The correlated anatomical facts have been studied and set forth, and it has been shown that the physical properties of the door do not change during action and that its action depends wholly on the pressure of the external medium (water) induced by the out-pumping capacity of the walls of the trap, first observed by Brocher, on the water-tightness of the door procured by the velum, as shown by Lloyd, and upon the actuation of a tripping mechanism also earlier described by the same author. There are no changes in turgor in the door cells and no reception or transmission of stimuli by the bristles, which are simply a part of the tripping mechanism.

(b) Evidence has also been obtained from a study of the flexures and movements of the door, and it is shown that such could not occur if there were changes in turgor. It is further shown that the histology of the door and threshold are correlated with the position, degree and direction of the flexures.

(c) It has been shown that during the flexures and movements of the door, there is no change in the position or form of the air contained in the intercellular spaces of the door, such as have been said to occur as a result of the movement of sap into these intercellular spaces.

(d) Killing of the bristles does not prevent the action of the door, although it has been postulated that the bristles receive and transmit stimuli to the tissues of the door.

(e) The rate of movement of the door during action is so rapid that, while it does not preclude the interpretation that it is an irritable response, it at least exposes it to grave doubt. Since the action can be repeated as rapidly and as often as the experimental conditions permit there can be no period of recovery. The action is so rapid that, if the mechanism is an irritable one, the latent period, or the period of transmission, must be very much shorter than any other case in the plant kingdom.

The conclusion is therefore reached that the mechanism of the door is purely mechanical, though this is not to say that the trap is purely passive, since the physiological activity of the walls has to play its part in exhausting water from the interior, as a reduced water pressure within is necessary for the action of the door.

A comparative study of 75 species of *Utricularia* supports this conclusion.

Foreword

In a paper already published in 1929, I gave a rather full account of the door mechanism of *Utricularia gibba*. This description was found to apply, except in regard to non-essential details, to most of the freely floating species, of which *U. vulgaris* is perhaps the most widely distributed and best known to botanists in general. In following papers I therefore did not use space for adding an extended description of *U. vulgaris* and its like. But most of the work of Continental students has been done on this species, so I have bethought

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myself that it would serve a useful purpose to give a fuller account of it. The recent publication of a view about the nature of the door mechanism quite divergent from my own, set forth in my 1929 publication, has supplied the occasion of combining an answer to that view with a description of some exactness, I venture to hope, of the anatomy, histology and functioning of the door mechanism, particularly of *U. vulgaris* and *U. intermedia.*

Such descriptions are difficult of achievement if we are confined to words alone, which, however adroitly used, put a heavy onus of constructive visualization on the reader. Diagrams are of much help, but a look through the microscope would be greatly better. The next best are good photographs, which, without any illusions about their necessary verity, can be used to make visualization relatively easy. To this end the present account is supported by a rather abundant supply of photographs each of which was made to clarify to the reader (as they have originally to the present writer) the point of description correspondingly set forth.

Introduction

There have naturally been two views of the nature of the action of the door of *Utricularia;* that, on the one hand, it is an expression of irritability, the bristles appearing to correspond to the sensitive hairs of *Aldrovanda* and of *Dionaea;* on the other, that the whole behavior is purely mechanical. The latter view has been supported latterly by Merl (23) (though with some caution), by Czaja (5) and by myself (18, 19); Merl, followed by Czaja, especially having investigated the mechanism by means of experiments designed to differentiate between the two types of mechanism. Although the issue seemed to be closed, I repeated to my own satisfaction many of their observations, and then proceeded to study the structure of the trap in the light of the physiological facts apparently demonstrated as true. I showed that previous notions as to the positional relations of the door to the threshold were incorrect, and further, that the water-tightness of the door is procured not by mucilage, but by the presence of a membrane, the velum, derived by partial exfoliation of the collective cuticle of the pavement epithelium, to use Goebel's term, in such manner that the resulting membrane remains attached to the forward three to five courses of cells of this epithelium (Plates III, IV, V). Later, I extended my examination to about 75 species of *Utricularia* and to *Polypompholyx,* from the results of which it became evident that the original account which I gave for *U. gibba* (Lloyd, 18) furnished a clue which enabled me to explain the minutiae of structure of these numerous forms. Taking all the varieties of structure into consideration, the impression was continually emphasized that the conclusion first experimentally supported by Merl was correct.

The contrary view, that the mechanism is an irritable one, in particular that the bristles, the touching of which discharges the trap, are sensory hairs and that the door and even much adjacent tissue are "motor" in nature, has been advocated by Brocher, by Ekambaram and by Withycombe (26). The two latter, curiously enough, supplied evidence which is antagonistic to their own notions, to be mentioned in detail beyond. It cannot be said that any

convincing evidence was advanced by any of these; rather it was a natural interpretation prompted by general knowledge. More recently, M. Kruck (17) has come forward in support of the same view, and advances evidence to support the contention that the bristles are in fact sensitive hairs, that these and the cells of the door are irritable and that the initial action of the bristle-door system consists in the reception, followed by the transmission of the stimulus by and through the bristles and through their bases to the cells of the door. It is claimed that in consequence the cells of the door contract, forcing water into the intercellular spaces, thus procuring the shrinkage of the tissues which effects a release of the door edge from the threshold, when the pressure of water completes the action. Kruck thus attempts to combine the indubitable fact that a reduced pressure of water, furnishing an unstable equilibrium, exists in the trap, as first observed by Brocher (1), with the view that the release of the door from its tight application to the threshold is an irritable response. This author further gives an account of the structure of the door and of its posture with relation to the threshold, accompanied by diagrams (Fig. 14) to make her meaning clear. Although it is quite thinkable that the door is an irritable mechanism, whatever be its posture, so that these two considerations must be regarded separately, nevertheless, Kruck has joined these two issues as part and parcel of her general contention. In what follows I shall consider first, the door; (a) its posture, (b) its structure; and secondly, the question whether it is purely physical-mechanical in its action, or is a sensitive-irritable mechanism.

Material

Kruck does not state the species studied by her, but from her sketches it was evidently *U. vulgaris* or a nearly related form. I have studied living material of *U. aff. gibba*, of *U. intermedia* and of *U. vulgaris* to the present purpose. All of these are in essential features, to all intents and purposes, identical.

Approach to the Problem

A general description of the trap or "bladder" should not be necessary here. A good one of *U. vulgaris*, though, in the light of more recent studies, faulty in certain respects, has been given by Skutch (25) in an article summarizing our knowledge to the date of his writing. Others are found in the papers of Merl, Czaja and Kruck. It need only be recalled that the trap of this type is a compressed hollow pear-shaped affair, with the door and threshold at the, as it were,

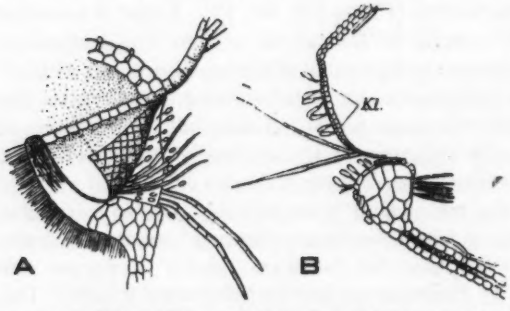

FIG. 1. (*A*) "*Median section*" *through trap of U. vulgaris. After Cohn* (*3*). (*B*) *U. flexuosa. After Goebel* (*15*).

truncated, narrow end, and supported on a stalk attached on one (ventral) side. The margin of the entrance is furnished with putative guides for prey, two antennae and lateral bristles, which need not be considered here. When the trap is in the "set" condition, the sides are concave (Brocher), as seen in Plate VI–55, and Fig. 4, due to the fact that the walls have the power of pumping out the water from the interior and thus lowering the water pressure within*. The greater external water pressure is exerted equally on the door, of course, but this is so constituted and postured that it can resist the pressure. The door is indeed water-tight (Brocher, Merl, Czaja). This property has been attributed to the firmness with which the door presses on the threshold (Brocher, Fig. 4, Czaja, Fig. 5) and to the presence of mucilage (Brocher). Before this water-tightness was known to be a fact, the door, since the time of Cohn and C. Darwin was thought to be a simple valve, under the edge of which minute animal forms could creep to their ultimate fate, often destruction. This seems indeed to be true of *Polypompholyx* (Lloyd, 20) but is not true of any of the 75 species of *Utricularia* studied by me so far. It is conceded, then, that the door is in a firm position of some sort, and requires some initial alteration so that the condition of unstable equilibrium (as Brocher first called it) may be disturbed. This initial alteration is procured in the species we are considering by some pressure on the bristles which, four to six in number, are inserted near the middle line of the door and near its lower free edge (Figs. 7, 13). Does such pressure constitute a "stimulus" which is transmitted by the protoplasm down the bristles, through their bases, and so to the cells of the door? Or does it merely cause a physical alteration of the shape of the door, so upsetting the unstable equilibrium? Not only has Kruck endeavored to answer this question in the former sense, for Ekambaram has also argued that this is the correct view, and has been at some pains to explicate the structure of the door mechanism and "sensitive hairs", but I think, incorrectly. Other and reasoned argument I know not of, while, on the other side of the question, as said above, both Merl and Czaja have advanced experimental evidence. Thus we are faced again with the alternative stated at the close of a previous paragraph.

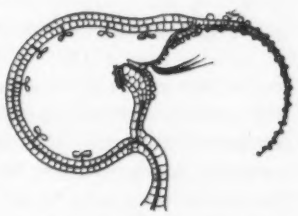

FIG. 2. *U. reniformis. After Leutzelburg (21).*

The Posture of the Door in Relation to the Threshold

By all observers, with the exception of Withycombe (26), the opinion has been held that the free edge of the door rests on the *top* or against the infacing *inner* edge of the threshold, and the figures when supplied leave no doubt about the meaning intended: Cohn (Fig. 1–A), C. Darwin, Dean, Goebel (Fig. 1–B), Meierhofer, Luetzelburg (Figs. 2, 3), Brocher (Fig. 4), Merl, Czaja (Fig. 5), Ekambaram. To the earlier, in whose opinion the door was a mere passive

**The properties of the wall have been extensively studied by Czaja (6, 7).*

valve, there appeared nothing more to say. After Brocher made his important observation, however, it became necessary to account for the water-tightness of the door. Brocher figured a blob of mucilage filling the entrance (Fig. 4) and postulated a compression of the threshold and door by the cramped-in sides of the trap. Others saw in the pressure exerted by the door edge on the top of the threshold (*e.g.*, Czaja, Fig. 5), coupled with mucilage, a sufficient explanation, the supposedly correlated structures of the door being specified. But Withycombe saw that such an explanation is inadequate. Studying paraffin sections, he thought that the forward edge of the pavement epithelium affords a resting place for the door edge, resisting its inswing (Fig. 6); he thought, further, to have observed a strand or mass of mucilage attached to the threshold, just in front of the position of the door edge, as postulated by him. While his idea has turned out to be right, his interpretation of the anatomy of the parts involved was incorrect. I have shown (Fig. 7) that the position of the door edge is such that it rests against (not in front of) the pavement epithelium either on a slightly outward-facing surface or (in various species) its mechanical equivalent (Plate IV–40, 41, 42; Fig. 7), that the door edge exerts a downward and inward thrust against a resisting middle zone of the pavement epithelium, and, further, that attached to the forward several courses (the outer zone) of the pavement epithelium there is a membrane, the velum, consisting of the loosened common cuticles of the cells composing it (Plate III, V–47), remaining, however, unloosed from the more forward cells (3 to 5 courses). This velum becomes so adjusted that it folds upward against the outer face of the door edge, thus sealing the slit between the door edge and threshold against inleakage of water*. The velum stretches across the whole front of the downwardly arched threshold as a veil (Plate VI). Since the extreme ends of the free door edge are attached to the *inner* angle of the threshold ends (*c*, Figs. 9–13), it follows, as is the fact, that the lateral extensions (*a*,*b*, Fig. 17–A) of the free door edge *must traverse the threshold*

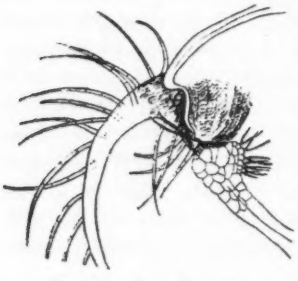

FIG. 3. *U. ochroleuca. After Leutzelburg (21).*

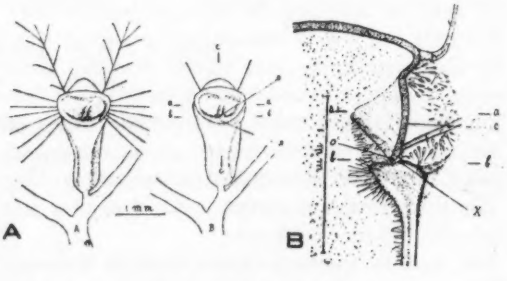

FIG. 4. *U. vulgaris. After Brocher (1). (A) His drawing recording his observation of the expansion of the walls on "stimulation". (B) The door mechanism.*

**I have pointed out elsewhere that a drawing by Giesenhagen (Fig. 8) made at the instance of Professor Goebel, of U. flexuosa, showed nearly correctly the velum, no reference to it being made in the text.*

to reach the front of the pavement epithelium (Figs. 7, 9 ,10, 13, *b*,*a*). Since the edge of the door which articulates with the wall of the trap articulates also with the ends of the threshold from the inner to the outer angle (*b* to *c*), it further follows that there must be a fold in the door, extending obliquely downward from the outer angle of the threshold (*c*) to the point where the door edge, having crossed the threshold, dips into position in front of the middle zone (*a*). The slit-like space or rather fissure thus formed between the door and the upward-turning threshold is a place where the water, under greater outward pressure, could easily gain entrance into the trap, were it not for the presence of the velum, which completely seals the front of the slit (Lloyd, 18). Thus we have an adequate explanation (*a*) of the ability of the door to resist the superior pressure of water on the outside, and (*b*) of the water-tightness of the door, in spite of the fact that the ends of the free door edge coincide in position with the inner angles of the threshold. Experimental proof will be given anon.

Kruck (17), however, thinks differently. The entrance to the trap, she says, is closed by the door—"dessen swach vorgebogener freier Rand von innen gegen ein Polster angelehnt ist"—quite the idea of Cohn (Fig. 1) so far as position is concerned. Her drawing accords with this statement (Fig. 14–1). According to this, when the trap is in the normal set condition, the free door edge is so placed that the bristles lie on the threshold, and the edge itself reaches inwardly over the inner margin of the threshold as far as the bifid trichomes. During the transmission of the stimulus which results in the initial opening of the door, the bristles change their position through an angle of about 100° (Fig. 14–2) caused by changes in the curvature of the door, from which, during the 15 min. required for recovery, regression to the original normal position occurs. Admitting the difficulties of direct observation mentioned by Kruck, it is still hard to see how she can have come to her conclusions. In order, however, to oppose objective evidence to hers, rather than merely to criticize, I have studied photographic silhouettes of traps in various conditions (before and after discharge, etc.) constructing diagrams therefrom. These will be discussed more fully beyond. Here I say only that an inspection of these diagrams, which are not intended to be complete, but only so far as to show the points at issue, namely the position of the door and bristles with respect to the threshold, will at once convince the reader that the free edge of the door cannot possibly reach far enough into the interior of the trap as to permit it *to lie against the inside edge of the threshold.* As to the exact position of the free door edge, I have already given evidence (18), but the point will stand further elucidation. I take the cases of *U. intermedia* and *U. vulgaris*, not previously dealt with by me in detail. The descriptions of the threshold by Cohn, Hovelaque and

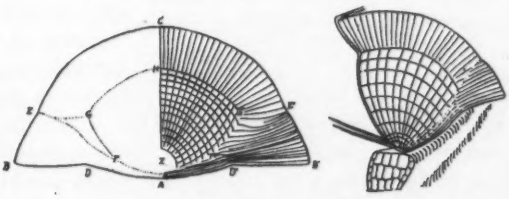

FIG. 5. *U. vulgaris. After Czaja (5).*

others have no cogency in this connection, for it is quite evident that the minutiae of structure escaped them, aside from other obvious errors of observation. The reason for this can be stated in Cohn's own words. Hitherto various observers "haben natürlich auch nicht vom richtigen Standpunkte die Organisationsverhältnisse aufgefasst".

The Threshold and its Structure in Relation to the Door

The threshold (called the collar by Darwin) is a raised-up arch of tissue, relatively massive, occupying the lower semicircle of the entrance into the trap. Of first importance in understanding its function is to understand the structure of its curved upper surface occupied by the pavement epithelium. If hitherto thought of any use in the mechanism, the component cells have been allowed to contribute mucilage to the sealing of the door to make it water-tight. It is, however, far more than this. It has become evident to me that the mucilage, undoubtedly present but not peculiar to this region, acts as a lubricator enabling the door, though pressing firmly on the threshold during the opening movement to slip over it with great ease. Beyond this its function is twofold; to provide a firm resting place for the door edge, opposing its inward urge under pressure of the outer water and to furnish, by the exfoliation of the common cuticles of some or all the pavement epithelium cells, the membrane which I have called the velum—a veil-like folded-over membrane, firmly attached to the outermost course of cells (Plate VI). In order to see this clearly, one must have recourse preferably to fresh living material, of which suitable sections must be cut through the appropriate planes (see Method). Unless the sections are truly normal to the axis of the threshold, its delicate curvatures are easily masked. When such truly orientated sections are obtained, the pictures afforded are as those seen in the accompanying figures (Plates III and IV). The pavement epithelium is seen to be divisible with respect to the trap as a whole into three transverse zones: inner, middle and outer, the "glandular" cells of which all have the three- or four-celled structure often described from Hovelaque on. The characters of these zones are best seen in a transverse section of the threshold through the middle point (Plate III, IV–40, 41,42; Fig. 9). The inner zone is composed of loosely packed glandular trichomes with capitals of oval form, one- or chiefly two-celled from between which protrude bristling plates, the remains of the enlarged and autolyzed cuticles. The middle zone is very compact of the capitals, here two-celled, flat-topped and dense of protoplasm and is usually somewhat arched. The outer zone is of looser

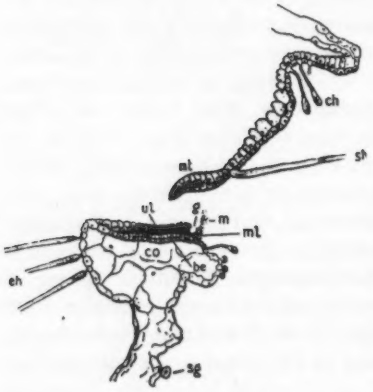

FIG. 6. *U. vulgaris.*
After Withycombe (26)

bacciliform unicellular capitals to which, for several forward courses, their cuticles remain attached as balloon-like enlargements. To the more forward of these there is attached a broad, irregular-looking membrane, the common cuticles of the middle and inner zones. In such sections as those seen in the figures, this membrane (the velum) appears lax and without orderly position but in a preparation in which the threshold is entire, and properly viewed, the velum rises up in front (Plate II–19) and curls over behind, as we observe in Plate VI–61, 62; III–31. The velum extends throughout the whole length of the threshold, from outer angle to outer angle; a slightly different appearance at the sides (Plate VI–57) being due to the stretching during the development of the trap, for the velum is set free before the full growth of the trap has been reached. It is this, indeed, which causes the velum to take its proper position as a stretched membrane to receive the front of the door edge when the door is normally closed. Otherwise it would be too loose and lax. Further examination of the section will show that between the velum and the compact middle zone there is a shallow depression into which the front edge of the middle zone dips down (Plate II–20, IV–40). The resulting forward facing surface is the firm resistant against which the free door edge rests. Without this, the trap could not function as an unstable equilibrium; and without the velum this is equally true. The following is experimental proof of this. (See Plate VI–55, 56, 57, 58). Choosing a well-developed trap, I discharged it by touching the bristles. With a very sharp small scalpel (see Method) I cut the velum at the side, whereupon the trap walls immediately expanded to their full and remained so, never recovering further exhaustion of the water, nor would the door again act to pressure on the bristles. This experiment I repeated several times. The figures show one example photographed before springing, after this and before operation and a half-hour after the cutting of the velum. Having carefully cut away the door to satisfy myself

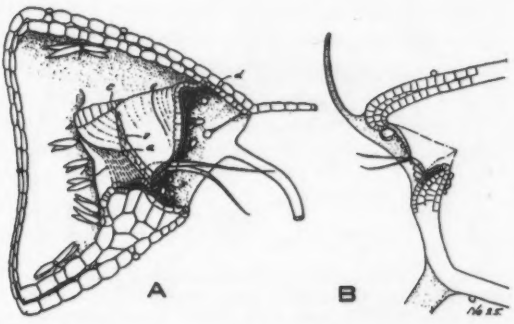

FIG. 7. *U. gibba. B, U. reniformis. After Lloyd (19).*

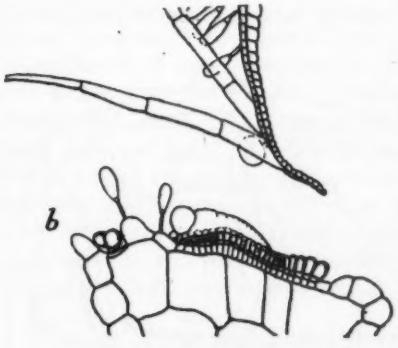

FIG. 8. *U. flexuosa, showing the velum (b). After Goebel (15).*

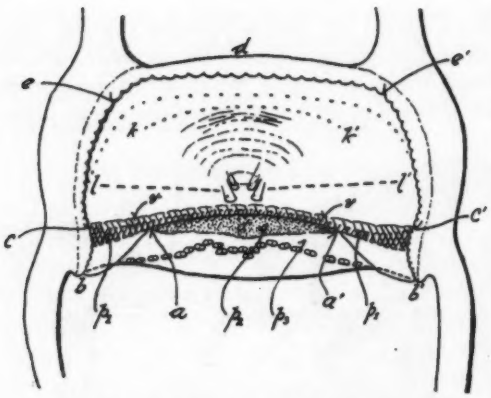

FIG. 9. *Door and threshold viewed with axis of vision normal to plane of threshold. a,a', the points at which the door edge leaves the front of the middle zone of pavement epithelium to cross over to the inner angle of threshold; b,b', inner angles of threshold; c,c' outer angles of threshold; e,e', points where the upper edge of door turns backward along sides of trap; l,l', line marking change of orientation of outer epidermis cells; k,k', limit between upper hinge and middle zone of door; d,d', median axis of door; p_1,p_2,p_3, outer, middle and inner zones of threshold; v, velum.*

that I had not damaged it, or any other part of the mechanism than the velum, I then photographed the latter on the operation side and on the untreated side, to show the tear. The operation is not easy, as the velum is tough and slippery, and it is difficult to hold the trap sufficiently to get the requisite purchase. A second example of a cut velum is shown.

Recurring to the pavement epithelium, we must further note that the zones mentioned are not coterminous with the threshold (Plate II–22). It already has been pointed out that the ends of the free door edge are attached to the inner angles of the threshold, each to each. As, in the middle stretch of the threshold, the door edge lies in front of the middle zone, in order to come into this position it must cross the threshold. This it does near the ends (Fig. 9). Here, where this crossing occurs, there is little or no compact zone, but a shallow oblique groove in which the door edge lies is evident (Plate III). Just at the point where the door edge emerges to lie in front, the threshold and its pavement epithelium are rounded transversely and the beginning of the compact zone is seen (Plate II–22). It is interesting to note that the physical opposition of the pavement epithelium as a whole is greater toward the sides (Plate III–26) than in the middle of the door; that is, the middle piece of the door is opposed by a lower obstruction to its inswing than on either side. The instability

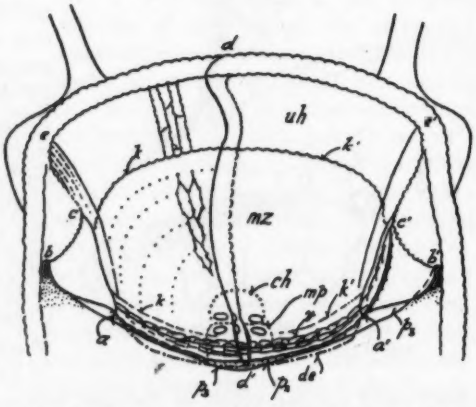

FIG. 10. *Same as Fig. 11, but with axis of vision parallel to the plane of threshold. Indices as in Figs. 9 and 17. uh, outer hinge; mz, middle zone; ch, central hinge; mp, middle piece (of door); de, edge of middle scallop of door lying a little below level of p_2. From c' to a', the broad dark line indicates the slit between the lateral triangles of door and threshold (exaggerated for visibility).*

of the equilibrium is therefore greatest in the middle, precisely where the release of the door edge, by the trigger action of the bristles, takes place. I shall indicate beyond that there is to this purpose a specialized organized portion of the door in the middle reach of the free edge. It is thus that the tripping action of the bristle-door mechanism is a very delicate one, but from experience one may certainly know that "die leiseste Berührung der Haare" (Kruck, 17, p. 260) may not be sufficient. With care, one may often touch the bristles, especially from below upward, without procuring response. But it would be idle to try to state how much pressure is required. I have observed large protozoa crawling about the door and on and about the bristles without any disturbance of the mechanism. Mere touch or slight bending of the tips is not enough. The best we can, indeed all we need to, say is that enough pressure must be given to the bristles to move slightly the middle piece of the door edge, and thus rob it of the even resistance offered by the pavement epithelium.

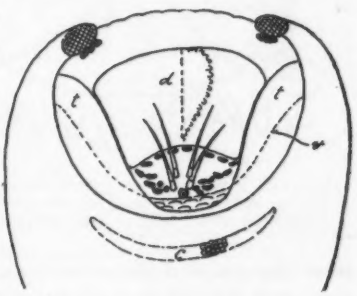

FIG. 11. *Door viewed from the front to show the distribution of trichomes on the front of the door (d) and on the sides of the entrance (t); c, zone of sessile trichomes as the edge of entrance (see Fig. 13) U. intermedia. Other trichomes in solid black.*

If the bristles are irritable, it would be reasonable to argue that mere touch regardless of direction would serve as a stimulus. But Czaja noted (1922) that the action ("reaction") of the door is made more difficult when the tension of the trap walls is overhigh; and (*b*) that the action is achieved more easily by pressure of the bristles downward than sideways, or, he might have added, upwards. I have many times verified the truth of these observations. It is well known that the sensitive hairs of *Aldrovanda* (Czaja, 8) and of *Dionaea* (Brown and Sharp, 2) do not require unidirectional stimulus. Brown and Sharp however found that in *Dionaea* release of the sensitive hair by two sudden partial releases from a previously bent position was unable to procure response; only bending the hair from its normal upright position is effective. It is thus seen that bending the bristles of *Utricularia* in one direction only might alone be effective. But we know that bending in any direction can be effective if in sufficient, though small, amount.

Form and Positional Relations of the Door

We may now consider the door with reference to some points of general structure. In view of the several attempts to describe this organ, it would seem here superfluous, were it not for the inaccuracies and inadequacies characterizing most of the presentations. Thus one author described the cells of the inner epidermis of the door as isodiametric, an error, though not an obvious one to a casual observer, as the appearances are deceptive. Kruck described these cells correctly with regard to this point.

In general form, the door when flattened out is roughly semicircular in outline, the free edge lying along the diameter (Figs. 17, 19). In its normal form when not forcibly distorted it is curved and corresponds roughly to a quarter spherical surface (Plate VI–59, Fig. 9) and it is approximately this form which it has when in its position guarding the entrance to the trap (Plate I; IV–38). Its articulation with the wall of the trap is again roughly semicircular, the two ends of the semicircle coinciding with the inner angles of the threshold (Figs. 9, 13). The plane, in which lies the semicircle of attachment, lies at an obtuse angle (*ca.* 100°) with that of the threshold (Fig. 13), being such that the chord of the arc formed by the door edge at its middle point lies near the forward edge of the threshold (Fig. 9). The free door edge is *too long to lie on the threshold along its inner edge and fit it* (Fig. 12). The normal position of the door edge is, as above said, with its middle reach against the forward edge of the middle zone of the pavement epithelium* and in order to reach this position the lateral reaches of the door edge must traverse the threshold. It is in this fact that we see the meaning of the shallow scallops characterizing the free door edge seen by everyone, and represented correctly by Czaja (Fig. 5) and by Kruck (Fig. 20). If what I have said be not true, then *the curvatures of the door edge have no meaning*, but this is far from the case. The middle reach, a low scallop, rests in front of the forwardly, slightly curved middle zone of the pavement epithelium (a, a', Fig. 9); each of two half-scallops ($a\,b, a'\,b'$, Fig. 9), or lateral reaches, lies obliquely across the pavement epithelium from the inner angle of the threshold ($b\,b'$) to a point approximately one-third of the length of the threshold on the forward edge ($a\,a'$). When the relative positions of door and threshold and their articulation with the wall of the trap are understood in the above sense, it is possible to map out the door into its regions with reference to the play of each during the operation of opening and closing. Czaja attempted this (Fig. 5) but erred in thinking that the outer surface of the door edge is pressed against the top of the pavement epithelium, and plotted an axis of flexure (E, F, F', E', Fig. 5) which does not exist. Kruck's description scarcely departs from that of Czaja and errs so far that she has, *e.g.*, drawn glandular trichomes attached to regions of the outer surface of the door where no trichomes can ever be found, while her drawings of the component cells are completely inadequate (Fig. 20).

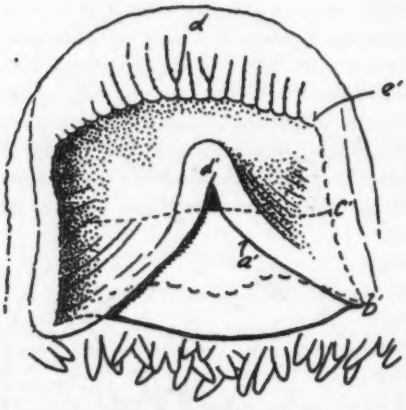

FIG. 12. *Diagram taken from a photograph of the door in the act of sliding over the threshold, in position of curve 2, Fig. 18, approximately.*

The position of the door edge against the forward edge of the middle zone and housed in the velum, can clearly be seen under a 16 mm. lens if the door is viewed "en face", since they lie close to a single optical plane (Plate II–19).

In Fig. 11 I have supplied a diagram to show the massing of the trichomes on the door and sides of the approach in *U. intermedia* which has a tuft on the forwardly facing surface of the door, much as shown by Goebel (15) for *U. flexuosa*, flanked by two tufts which arise from the walls of the approach and closing access to the spaces formed between the lateral parts of the door and

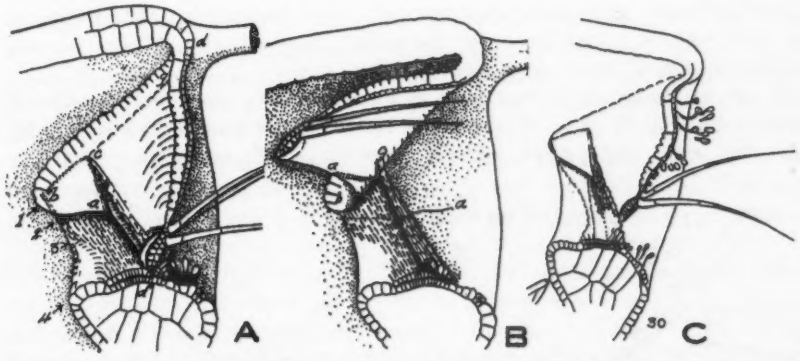

FIG. 13. *A and B, diagrams of sagittal section of door apparatus of U. intermedia, A, the door in closed position. The position of the middle piece as in curve 1, Fig. 18, is indicated; B, door in open position, approximately. The velum is now lax. C, sagittal section of U. flexuosa. The door is raised above its normal position. Drawn from a photograph without readjustment, to show better the velum in lax condition.*

trap wall. Together the three tufts form a shallow funnel leading to the base of the bristles, the effect of which is to reflect the errant motions of prey toward the bristles. The drawing made by Skutch (his Fig. 1, 25) shows the relations of these tufts as viewed laterally. The specific structure of the trichomes was detailed by Meierhofer (22).

If we look at the door *in situ* along an axis which passes through the middle of the door at right angles to the plane of the threshold, we can get a true picture of the relation of the door to the trap (Fig. 10). Below we see the contour of the threshold, a somewhat flattened inverted arch, springing from a cushion of cells, one each side (*b a a' b'*). This cushion (just above *c* and *c'*) is the edge of the door. Above appears an arch (*e d e'*) of greater diameter, extending between the bases of the antennae and somewhat beyond. This is the articulation of the middle of the upper edge of the door with the wall of the trap. From the antenna base on each side, the articulation turns backward in a circular

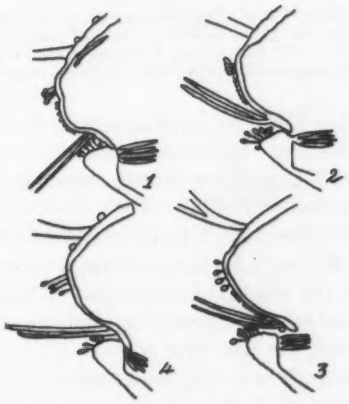

FIG. 14. *Diagrams copied from Kruck's figure to show the supposed changes and curvatures of the door during response (Kruck, 17).*

sweep to the forward angle of the end of the threshold (*e c b*, Fig. 9). From the forward (*c*) to the inner angle (*b*) of the threshold end, the door edge is raised a little away from the wall by the end of the threshold, so that the attached door edge here turns in slightly and, as it crosses the end of the threshold, it turns again to face obliquely the interior of the trap. In effect, the door is cramped in to fit a narrower arc than that which it would, if free, normally occupy, as Brocher apprehended. This makes for a close fitting of the door edge as it traverses the threshold. Luetzelburg (21) showed this detail in his Fig. 47 for *U. ochroleuca*.

If now we dissect away the door, in water, we may map out the areas of significance (Fig. 17–A). When freshly removed, the door retains its curved form, but with more spread, and the outer hinge cells curve more strongly.

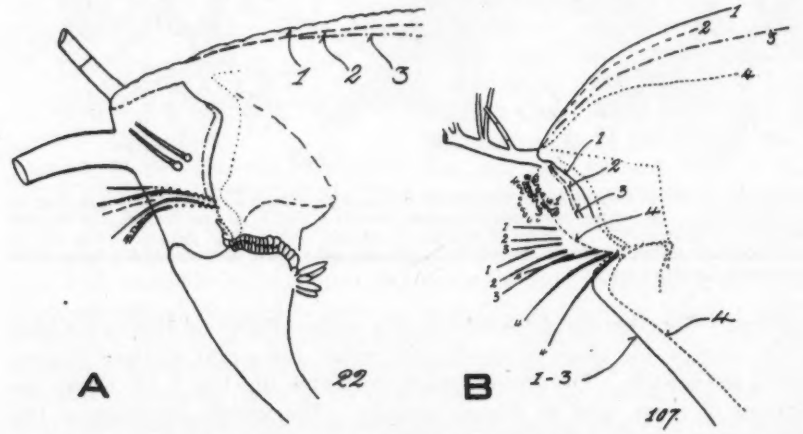

FIG. 15. *A, Diagram from projected images of U. gibba reproduced as Figs. 1–3, Plate I, to show changes in posture of door and walls; 1, before discharge of the set trap, 2, immediately after, and 3, after puncturing; B, a similar set of diagrams for U. vulgaris the additional curve 4 indicating the position of door etc., after making a sagittal section.*

This happens immediately and is owing to the turgor pressure of the cells added to the natural elasticity and cramp of the tissues. Plasmolysis with 0.5 *N* potassium nitrate results in flattening them out to some extent, but by no means entirely (Plate II–16). We shall refer to this again. By killing the door with KI-iodine and subjecting it to slight pressure, the door may be sufficiently flattened out for observation. Photography of it is difficult because of the residual curvatures. When flattened, we may arrive at the following analysis. The roughly semicircular form of the door (Fig. 17–A) is bounded along the free door edge (*b b'*) by a curved margin, having the form of an archer's bow of a more ancient sort. The middle scallop (*a a'*) occupies somewhat more than one-third of the length; the end scallops (*a b*, *a' b'*) each less than one-third. The middle scallop lies against the front of the middle zone of the pavement epithelium, itself bowed slightly forward to their mutual fit (Fig. 9). At *a* and *a'*, the door edge passes obliquely inwardly across the threshold, so

that the triangle *abc* lies against the end of the threshold (and mutually *a'b'c'*). The point *c* lies at the outer angle of the threshold, *cb* coinciding with the end thereof. The broken line, *ac* (Fig. 17), indicates a fold in the door, fitting the forward edge of the threshold, from its outer angle to the point where the door edge emerges to the front of the threshold. Thus, from *c* to *a* there is a re-entrant space, a mere slit between the face of the door embraced in the triangle *abc* and the corresponding face of the threshold (*a'c'*, Fig. 10). This is proved by the above-mentioned experiment, in which, by cutting the velum, *c c'*, which lies in front of this re-entrant angle, water is admitted and the trap is no more able to set itself (Plate VI). This justifies marking of the triangles in question, *viz.*, *abc*, *a'b'c'*.

Since *ce*, *c'e'* are those parts of the door edge which are attached to the lateral walls of the trap, there is a rather gentle fold, *ea*, *e'a'* between the lateral and frontal aspects of the door. There is, however, no structural correspondent. That area which remains, *e a a' e'*, is the front-looking part of the door. In this there are two critical areas (a) a roughly circular area, the *central hinge*, the thinnest part of the door just above the bases of the lower pair of bristles; it is a hinge allowing principal movement on the axis, *1*, *1'* (Fig. 17–B), and (b) a much thickened part, with very much thickened outer walls, the *middle piece* (Plate VI–59, 60) delimited by the broken line *i i'* (Fig. 10, 17–A) which rotates backward on *1 1'* (Fig. 17–B) when pressure of the bristles supervenes. This is the tripping action. Beyond the area enclosed by *i i'* on either side the door edge remains thickened, thinning but little toward *b* and *b'*, not having, however, the special strengthening derived from thick outer walls, shown in Figs. 51, 52 of Plate V.

Flexures of the Door during Opening and Closing

We shall better comprehend the histology of the door from the point of view of function if we understand the flexures which it has to undergo during movement. Ekambaram has already told us about this so far as the introverted position is concerned. The door areas are mapped in Fig. 17. First we note that the initial movement which frees the door is a slight upward movement of the middle piece. This can result from any movement of the bristles in any direction, though it is generally conceded that upward movement is least effective. But although upward movement of the bristles is conceded to be not so effective, it

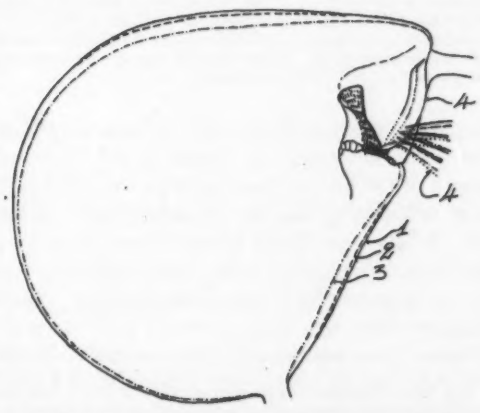

FIG. 16. *The same as Fig. 15–B, for U. intermedia, from projected images reproduced as Figs. 7–10, Plate I.*

does, as a matter of fact, work. We may be correct in explaining this by supposing that the middle piece, being moved slightly outward, releases the door edge on either side, which would be nearly as effective. At all events, the movement must be such as to release part or all of the free edge of the middle piece from its resting place against the middle zone of the pavement epithelium (Figs. 7, 13). This being accomplished, the door is free to move inwardly in front of a column of water which will be great or small according to the extent of exhaustion of water from the interior of the trap. Assuming a maximum, this column of water pressing equally everywhere on the door pushes it inwardly as a whole. Inasmuch as the free door edge is longer than the threshold (Figs. 9, 10, 12) over which it must slide, it must

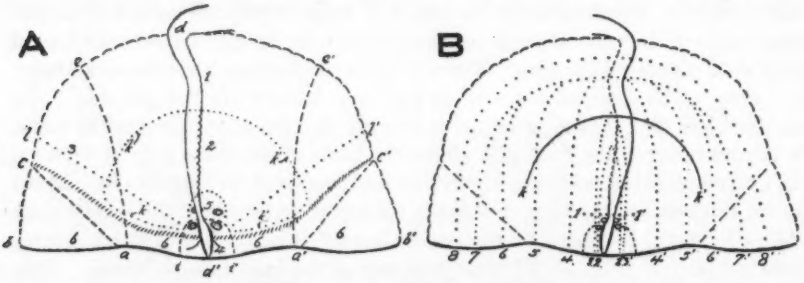

FIG. 17. (*cf. Plate II–21*). *A, plan of door laid out flat, U. intermedia. a, angle between middle and lateral scallop of door edge; b, position of inner angle of threshold; c, of outer angle of threshold; d d', median axis of door with outline of sagittal section of door overlaid; e a, approximate position of flexure between front triangle and backward sweeping sides of the door; l to centre to l', line indicating the change of orientation of the outer door epidermis cells from transverse (with respect to radii of door) to longitudinal at the sides (l b i); k k', a closed line inclosing the middle zone (2) and separating it from the outer hinge (1); 5, central hinge; 4, middle piece; 3, identifies the area shown in Fig. 21–A; 6, identifies an area, the edge zone, a part of which is shown in Plate III Fig. 34. This is not a sharply defined area, but is more properly regarded as a continuation of the outer hinge. Primed numbers are mutual with unprimed. The line of the velum edge as it is applied to the door when normally closed is indicated by the hatched line c c' (see Plate II–19 and especially Plate VI–63). B, Diagram to indicate approximately the flexures of the door during its inswing on discharge. 1 1', the axis on which the middle piece swings in clearing the opposing edge of the threshold middle zone to come into position 1, Fig. 18. This is not a constant axis, as touching the bristles laterally, etc., causes flexure of the circular central hinge in the corresponding axis, and this has the same effect. Curve 2 in this Fig. 17–B corresponds to curve 2, Fig. 18, curve 6 to curve 4, and curve 8 to curve 6, approximately.*

therefore buckle at some point. This is always at the middle point and nowhere else. By measuring the length of the inner surface of the threshold and the length of the line of the door edge we arrive at the ratio 100 to 110, the threshold being 10% shorter, approximately. Using this measure, the Curve 2, Fig. 18, has been drawn (using a bent wire as a model). To adjust itself to this buckling, the door must curve, this being a matter of observation (Fig. 12). As the edge of the door moves inwardly, the middle piece with its adjacent length of door edge tends to revert to its natural curvature, but since the water column is pushing inwardly, the curvature is reversed and the form of the door will then assume curvatures somewhat as 4 and 5 successively. Since further the water column is now pressing, as if it were a solid rod, on the door radially, the door is opened farther till it assumes the form of Curve 6 in the same

figure; and, as the surface of the door is not that of a true sphere, but is spheroidal, bellying outwardly, it must, during the passage inward, reverse its curvatures, becoming hollowed in the opposite direction from normal. This takes place on passing from Curve 3 to Curve 4, approximately. The introverted position of the door is seen in the now inwardly curved middle zone and middle piece and the position of the latter is such as to swing the bristles up clear of the entrance. They seem to lodge in the tuft of projecting trichomes projecting from the door surface. Ekambaram likened the form of the door at this position to that of a boat, the bristles lying in the hollow like oars. Since he was observing an introverted door which held in that position after some manipulation, it would assume the form of Curve 6*a*, as I have frequently observed. This cannot be the form of the door as a strong column of water is passing in, especially as the sides of the door have in this position little resistance. A feature of this change of curvature which is not appreciated easily is that which happens because of the sharp change of direction of the upper hinge as it curves back on either side from the transverse reach, as very well seen in *U. gibba* (*e'*, Fig. 12), namely, there is a tendency of the fronting face of the door to work as a unit, namely, *e a a' e'*, Fig. 17, so that along *ea* and *e'a'* there is a maximum of distortion.

Inasmuch as direct observation, because of the swiftness of motion during normal opening, is not possible until slow motion cinematography can be brought to our aid, it is natural to follow the behavior of the door experimentally. This can be done by pushing in the door with a blunt needle; or the door may be made to move by slight lateral pressure on the sides of the trap, this meanwhile lying on its side under observation. An initial pressure of the middle piece is required, possibly a little further pressure to get the door started in the right direction. Doing this one may easily learn that it requires very much more pressure against the belly of the door to push it inward than upon the middle piece. Ekambaram's estimate (11) was obtained by pressure against the middle of the door. This is merely an indication of the amount of pressure the door can withstand when pressed upon locally. When pressed upon by the front of a water column, its effective resistance must be greater. The pressure is distributed outwardly to the edges of the door, tending to make the curve of the hinge zone deeper, and compelling the door edge, especially the middle scallop (*a a'* Fig. 17) to thrust against the pavement epithelium. This thrust must be firm and the door edge must not bend as supposed by Czaja

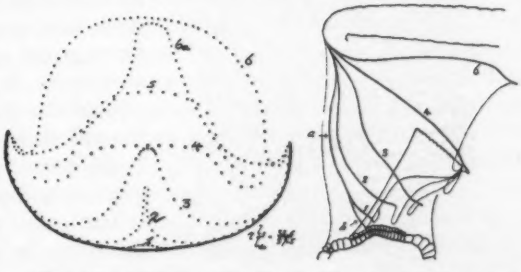

FIG. 18. *Diagrams to indicate approximately the curvatures of the door as seen from the front or back, and laterally, the sagittal profile of the door only being indicated. The upward sweep of the middle piece is the first movement (1). Then follows the sharp flexure of the door edge through the middle of the middle piece caused by the greater length of the door edge over that of the threshold. Introversion occurs between 3 and 4.*

(Fig. 5) and as implied in other descriptions. This thrust is not shared in by the door edge from b to a and b' to a', since here the edge is directed inwardly against nothing at all. Instead, the thrust is taken up by the door along the lines $a\ c$ and $a'c'$, against the pavement epithelium, the curvatures of which conform to this duty (Plate III–26). The whole line of thrust therefore is $c\ a\ a'\ c'$ against the forward edge of the pavement epithelium, but inside the outer velum bearing zone. Obviously $c\ a$ and $c'a'$ are not straight lines as shown in Fig. 17, but here appear so, due to the exigencies of drawing a flat plan of the door. These two reaches are peculiar in that they are weak places from the point of view of water-tightness of the door, only possible by the presence of the velum, as I shall show beyond.

After the inward swing of the door is complete, as the inrushing column of water commences to lag, the intrinsic qualities of the door make themselves felt once more. The position of the door is reversed again, *against the still forward moving water column.* This Brocher, followed by others, showed by puncturing the trap with a needle after it had been sprung in normal fashion. The sides of the trap then still remain concave after normal discharge (Plate II–17, 18; VI–55). This however may not happen if the door has lost its turgor, as then it does not always have a residual elasticity sufficient to the purpose. When a trap dies, the last part to succumb is the door. I have examined supposedly dead traps in numbers and have usually found that, though the walls are quite dead and fully expanded, some of the door cells are still alive. A trap which is in a moribund condition, too much so to exhaust water, may still be made to work by gently pressing out the water which escapes under and around the door edge, but only if care be taken not to push out the door too far so that the velum is caught inside. When the door is dead it can no longer exercise a strong thrust against the threshold so that when one experiments with such an one, pressing out the water, on gentle release of the pressure the door may give way at once, no snap action being possible. But it is a nice question how far the elasticity of the cell walls of the door alone contribute to the action; for I have noticed that when the door is quite dead, the cell walls have undergone autolysis in the outer hinge zone especially, thus weakening the whole structure.

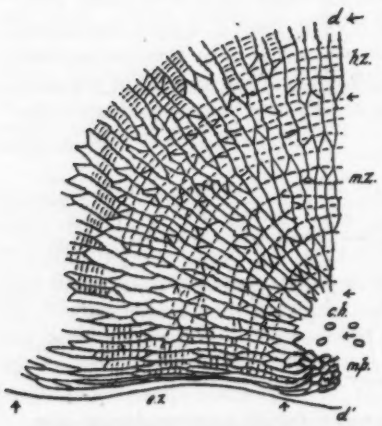

FIG. 19. *Diagram of cell structure of the inner face of door based on original preparation reproduced in Plate II–21. The structure of the central hinge is not indicated; see Fig. 23.*

The total behavior of the door therefore is that of a highly elastic and flexible curved plate, and quite different from what it would be were the door to any extent deprived of these properties, as happens when the door loses turgor.

Histology of the Door

It will now be convenient to consider the histology of the door. Its curvatures are simply and better understood by referring in the diagrams (Fig. 17) to the superposed profile of a sagittal section. The effect in a mechanical sense of these curvatures is to offer a convex surface to the hydrostatic pressure greater without than within.

The door is constructed of two courses of cells only, as has long been known, the outer and the inner epidermis. As Meierhofer showed, the door is anatomically a continuation of the wall, itself two cells in thickness, except in specialized regions, *e.g.*, in the threshold and along the vascular tracts.

The outer course is composed of shallow cells with sometimes curved but more often zig-zag lateral walls (*i.e.*, those normal to the surface of the door) and at each angle there is a rod (normal to the surface of the door) (Cohn, 3) giving such support to the lateral walls as to enable the cells to resist collapse under bending (Plate V–43-46; Fig. 21). These cells are capable of only very

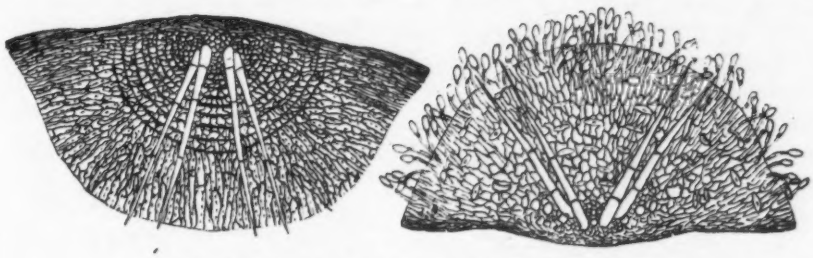

FIG. 20. *Inner and outer faces of the door after Kruck.*

limited stretching, even under maximum turgor, because of the much greater area of the tangential walls. In the middle triangle of the door there are two regions slightly distinguishable by the character of the shapes of their cells. Those in the outer hinge area are nearly isodiametric and have a zone of cells with a large re-entrant angle formed by curved walls (Fig. 21–*B*) while those opposite the middle zone are elongated crosswise the door, so that their long axes are normal to the long axes of the cells of the inner course (Fig. 21–*C*). The pattern of the middle triangle of the door merges laterally with that of the two lateral triangles (lib, Fig. 17–*A*) where the cells are arranged with the longer axes radially placed, the central hinge being the centre, as Ekambaram showed (Fig. 22). These cells have much simpler and straighter walls, from which angles and rods are absent (Fig. 21–*A*). In the area covering the middle zone (*k k'* 5, Fig. 17) there is scarcely a trace of curvature in the walls, they being strictly zig-zag. If under the stress of turgor there is a change in the dimensions of these cells, it must be the greater across the door, namely, periclinally in the middle triangle and anticlinally in the lateral. Whether great in amount or not, this stress opposes itself to that in the cells of the inner course to make the door capable of very considerable outward curvature (Plate II–14, 15). As the central hinge is approached, the cells become very small,

nearly isodiametric and relatively more richly supplied with rods, which form an intricate symmetrical pattern (Fig. 23–*B*). In the middle piece of the door edge the pattern becomes more evidently bilaterally symmetrical, the cells, which have very many and thick rods, gradually merging into the elongated cells of the door edge, which are really part of the lateral triangular regions. Photomicrographs showing small characteristic areas are given as Fig. 43–46, Plate V.

The function of this course of cells, as just briefly indicated, is analogous to that of the metal strip with the lower coefficient of expansion in a metal thermometer. This is shown by the fact that on free bending of the door this layer always tends to take the convex side as occurs especially when a narrow strip from the middle of the door is cut and laid in water (Plate II–14, 15). In any case the amount of extension or of bending of this layer is normally always small.

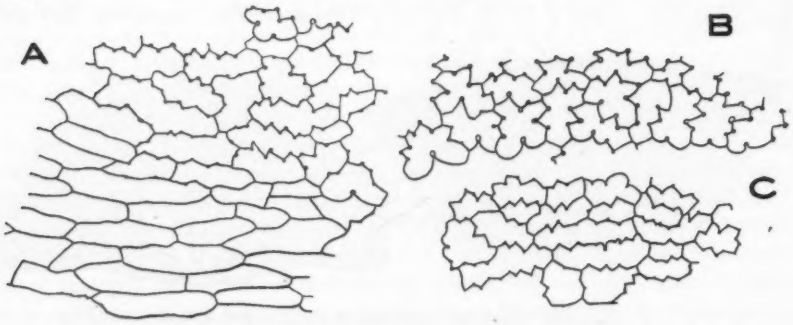

FIG. 21. *Outer epidermis of door. A, diagram of cell structure of area near 3, along l l', Fig. 17; B, of area 1, in the same figure and C of area 2 in the same figure. Compare with Plate V—43–46.*

The outer surface of the door is always conspicuous for its armature of trichomes (first well described by Meierhofer, 22) of which we are here concerned however only with the bristles or tripping bristles as I have called them. These in the species under consideration are usually four in number, abnormally as many as six (or seven, Darwin) arising as four (resp. more) outer epidermal cells arranged at the angles of a trapezoid which bridges the juncture between the middle piece of the door edge and the centre hinge (Fig. 23). The bristles are gently and elegantly tapering from the base to the extremely tenuous apex and are more or less curved, the lower pair the more. They are normally three-celled, morphologically equivalent to the trichomes in general, though they have been represented by various authors as having four to six cells. The emplacement and spread of the bristles are such as to make them the long arm of a lever with the fulcrum at their base, the weight being represented by the middle piece of the door. (Plate I–5; IV–38; V–51; Figs. 24–*A*, 24–*C*).

The inner epidermis is far different. The inner door area consists of the outer hinge (Fig. 17–*A*, *e k k' e'* and the lateral extensions), the middle zone (*k k'*), the central hinge (5), the middle piece (4) and the edge zones. The

general arrangement of cells is distinctly radial (Fig. 19; Plates III, IV, V) from a centre in the central hinge. We begin with this. Its cells are very small in all dimensions, but only relatively deeper. The walls are thin, but the large rods serve to support them under the considerable bending they have to suffer; and correlated also with this are the many infolds of the tangential walls, more obviously the outer, in which they occur not only concentric with those of the middle zone, but obliquely, thus adding to the flexibility of the tissue in all directions. As the central hinge merges into the middle piece, in the tissue lying between the upper pair of bristles, the infolds all take a single direction, parallel to the sagittal axis of the door, in conformity with its subjection to the strong longitudinal flexure which takes place when the door is sprung (Fig. 12). The infolds, as of course the rods, are here very numerous and very regularly placed.

In the middle piece the cells are arranged in curves swinging into the edge zone (Plate IV–35) and so on to the outer hinge zone. The pattern is bilaterally symmetrical, merging with the pattern of the outer course of the middle piece which is distinctly bilateral. The very small cells are provided with very numerous plate-like rods lying generally parallel to the line of flexure. The outer walls of these cells are very thick as is the case also of the contingent cells of the inner epidermis.

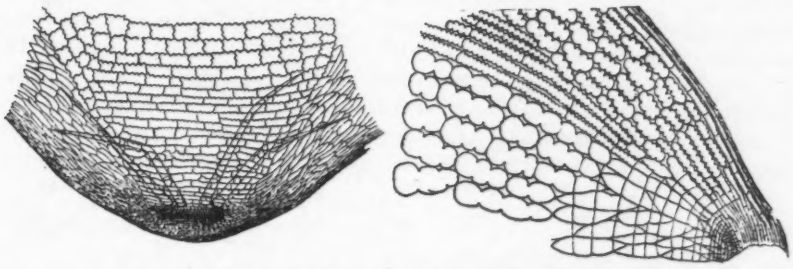

FIG. 22. *Outer and inner epidermis of door after Ekambaram.*

The central hinge is a very small circular area which merges upward and radially with that of the middle zone, the whole radial extent of which is shown in Plate IV–35. Here the cells are much larger and while roughly spindle-shaped (incorrectly described by Czaja as isodiametric), they display much irregularity of form (Fig. 23–*A*). Their long axes are always radial in position. The side walls, those normal to the surface of the door, are supplied with rods (Goebel, 1891) which occur in periclinal lines, rods which, often cylindrical, may also be irregular plates (Plate V–54) often but not always disposed periclinally. At these the walls usually show "broken joints"—they do not run straight from one to the other side of the rod—this condition often giving rise to quite peculiar appearances. In effect it looks as though the portions of the walls between the rods had been accidentally displaced (Plate IV–35, 39). But the observation which has always pinned the attention is the appearance of circular lines approximately equally spaced, traversing the

long axes of the cells (Fig. 19; Plate IV–35, 37, 39) and centering on the centre of the central hinge. These have been regarded as cell walls (Withycombe, Ekambaram, Czaja) but are not (Goebel, Meierhofer, Kruck). These circular lines have been thought also to be confined to the middle zone, but this again is not true. Here they are more widely spaced radially, are fairly regular, though their regularity has been exaggerated, as *e.g.*, by Kruck (Fig. 20) and convey the impression of concentric circles. In point of fact, the lines are everywhere present but much more numerous, less regular and far less

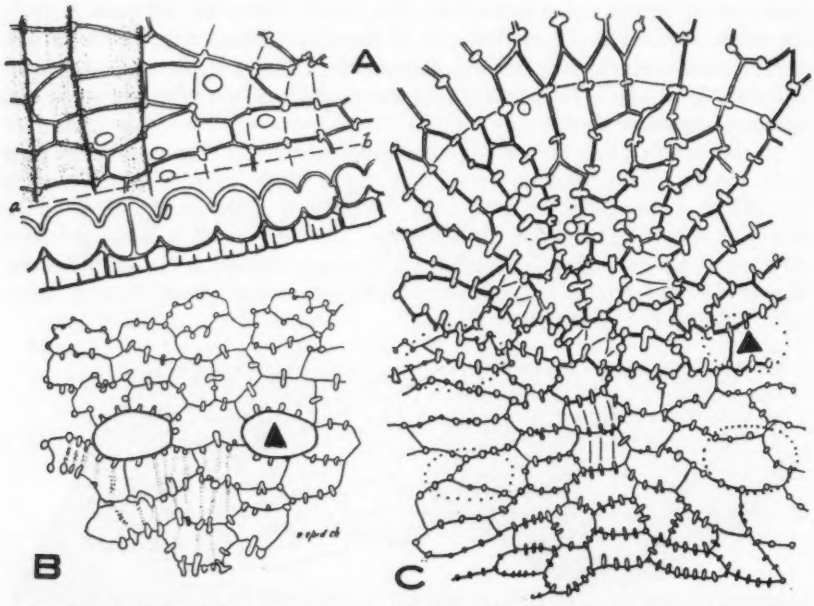

FIG. 23. *A. A few of the cells of the inner epidermis of the middle zone to indicate to what is due the appearance of circular lines (see Plate IV—35, 39; Plate V—48). A section running through a–b is shown alongside. To complete the diagram the cells of the outer course are also shown; B, outer epidermis cells in the central hinge (above the bases of the upper pair of bristles) and a portion of the middle piece (below the bristles), cf. Fig. 46, Plate V; C, the inner epidermis of the central hinge and of the middle piece (in part) to be compared with Plate IV—35, 36. In a few of the cells are shown the infolds of the outer walls running hither and yon in the central hinge (i.e., above the dotted-in bases of the upper bristles) and longitudinally (parallel to the sagittal plane) in the middle piece.*

conspicuous in the outer hinge zone and in the edge zone. The effect is, in short, produced by a roughly circular *corrugation of the door surface*, to which however there is a corresponding set of corrugations on the inner (tangential) walls of the outer epidermis. Each corrugation runs athwart the radially placed cells without regard to the positions of those cells, but always with regard to the positions of the rods, so that if we plot the rods on a diagram, we have indicated the course of the corrugations.

Regarding a single cell, *en face*, it is roughly spindle-shaped but may have a truncated end (Fig. 23–*A*). In the lateral walls there are rods which are

placed in pairs one on each side of the cell in the periclinal direction, each one of a pair being the mate of a pair in the neighboring cell, and so on from cell to cell. The outer and inner walls running radially between two pairs of rods are curved like a cylindrical lens, so that running periclinally between the outer ends of each pair of rods there are infolds of the wall (Figs. 23, 24). Since the outer wall is thick and supplied with cuticle, the infold here is optically conspicuous and appears, at an appropriate focus of the microscope, as a bar connecting the tops of the rods. The apparent bar together with the two rods looked to Cohn like ring thickenings, but Meierhofer dissented. Although Kruck realized that a cell wall is not in question, yet thought of the infolds as true bars which, with the rods, form U-bars or U-shaped thickenings.

If now we examine Fig. 24–*A* we shall see why the prominent circular lines appear as they do. The door is of course more or less curved so that on microscopic examination the optical plane will be parallel to the tops (anticlines) of the corrugations in one place (arrow *a*) but, as the surface of the door is tilted to the eye, the axis of vision will lie as arrow *b*, Fig. 24–*A*, and when tilted still more the axis of vision will lie as arrow *c*. When the axis of vision is as arrow *a* the image cannot be sharp and as the focal plane is moved up from 1 to 2, the image splits into two ghostly images, sharper on one (the cuticle) side than the other. When looking along axis *b* one looks through a thick layer of the cell wall. It is this which gives us the circular lines. It is true that when looking along axis *a* we can get an image, but it is relatively faint and ill-defined, which always breaks into two symmetrical images on raising the focal plane. There is therefore no structural unit, such as a bar; there is only an infold of the wall. In Plate IV–35, 37, 39 one can see all the various appearances thus obtainable. Various published drawings give the impression that these lines are continuous circles (Fig. 20, *e.g.*) whereas they are never so,

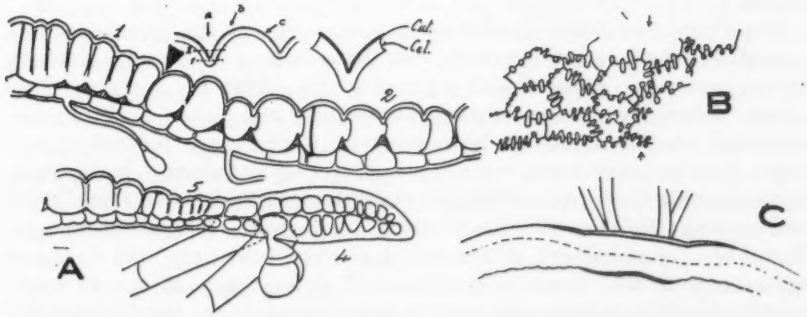

FIG. 24. *A, Diagram of longitudinal sagittal section of the door including, at 1, the lower part of outer hinge (ending at pointer), (2), the middle zone, (5), central hinge and (4), middle piece. The numbers along the section refer to the areas indicated in Fig. 17–A. Above: a, b, and c with arrow points, indicate axes of vision which afford various interpretation of the bellows structure; on the left of this, the outer wall to show the cellulose wall cel., with its cuticular investment, cut. B, A small area of the outer epidermis of the middle piece near the door edge to show the numerous rods. C, optical section of the middle part of the door edge to show the middle piece with its thick epidermis and the paired bulbous swellings one on each side of the midline. Compare with the photographs, Plate VI—59, 60.*

since they bifurcate and become more numerous as the door edge is approached and even in the middle region they similarly bifurcate.

The total effect of the corrugated inner and outer walls of the cells of the middle zone, as indeed of the hinge zone, is that of a bellows, thus appropriately called by Ekambaram (1916). The inner epidermis therefore is capable not only of being twisted but of much expansion and compression, the former being normally active, the latter passive. The tendency to expansion is ever present in the living door to bring it into its proper position when, after tripping, the door has been pushed in by the pressing column of water.

The outer hinge zone (between *k k'* and *b d b'*, Fig. 17–*A*) which occupies a width of about one-third of the longitudinal depth of the door, widens suddenly as it approaches the door edge, where it merges with a zone, the edge zone (Fig. 17–*A*, 6) having about the depth of the middle piece. The histological character of the edge zone is like that of the outer hinge zone (Fig. 19) and they will be considered together. Here the cells are longer and have more nearly transverse septa but in the edge zone, oblique; there are many more and more closely placed rods and correlatively more infolds of the outer wall. These cells exhibit the maximum capacity of bending or stretching the outer walls (Plate II–14, 15) and can turn the door outward through a wide angle (about 120 to 140°). The cylindrically curved sections or bellows structure of the outer and inner tangential walls are principally concerned with this property (Fig. 26).

The lateral walls of all these cells are flat and run straight between rod and rod. From bay to bay the direction may change somewhat, so that the radial lateral cell walls are variously placed within limits. The small amount of stretching normally imposed on these walls would be easily taken up by them. I am unable to confirm Kruck in stating that between the cells there are pectin masses.

Some published drawings of these cells show them with strongly curved, spring-like *lateral* walls between the rods (Ekambaram), while other observers having seen them, speak of such walls (Fig. 22). This is a mistake. They appear thus in the door when it *has been removed and allowed to lie in water*, especially when flattened out for observation. Soon, near the cut or torn edges, those entire cells next the cut or tear take up water and bulge laterally, at the expense of and encroaching on their (damaged) neighbors (Plate IV–35) and in a short while the disturbance spreads till an entirely false picture obtains. If a door is removed and photographed at once, before the cells have had opportunity to take up water (or better still, *in situ*) the walls are all nearly straight (slight curvatures only may be seen), except at the ends of the entire cells next or near the cut edge. If the door be immediately plasmolyzed or killed with iodine, such bulging does not supervene (Plate II–21; Fig. 19). The walls now, however, are lax, and this equally does not occur in life.

Kruck has stated that the cuticle of the door is not absolutely tightly applied. To this I cannot subscribe. Sometimes when a preparation lies in water, the cell walls of the door become rapidly hydrolyzed and the cells consequently completely disorganized, setting the cuticle free. Cases of this kind offer in

my experience the only suggestion of what Kruck may have seen. There is, in fact, indicatory evidence that the cellulose of the walls of the door cells is different from that of the walls in general (Lloyd, 19) though at the moment I can do no more than offer the above evidence, to which, however, may be added that the extremely elastic character of the door suggests that the cellulose is of the nature of that of collenchyma. Preparations stained with Sudan III demonstrate a tightly coherent, delicate cuticle everywhere on the door surface. Sulphuric acid sets it free.

Summarizing the above, it becomes evident that the door is, as generally stated, a strongly elastic structure, bending readily not only along its radial "static" lines (Czaja) but in any direction. In a condition of turgor the tissue exerts its maximum bending outwardly, never inwardly, with respect to the trap interior (Plate II—14–16). This maximum bending results from the strains inherent in the cell-walls, plus the turgor pressure. When plasmolyzed, thus subtracting the turgor pressure, the door *still bends outwardly*, though not as much, making now an angle of about 45°, with its enforced position when attached normally. The inherent curvatures are owing to the fact that the extensibility of the outer door epidermis is less than that of the inner epidermis with its bellows structure. The construction is such that the door continually strives to take a position outwardly beyond that which, by virtue of a *force majeur*, it is compelled to take; that, namely, in the living, uninjured trap. It can take no other position except as the pressure of the outer water affects it on tripping the trigger mechanism, which is the bristles and thickened middle piece of the door edge. The mobility of the door is the result of the bellows-shaped inner and outer walls of the inner epidermis.

The Door Regarded as an Irritable Mechanism

We may now consider specifically that view advanced recently by Kruck, earlier by Ekambaram, and examined by Merl experimentally. Merl concluded against the idea that we are here dealing with an irritable mechanism and Czaja agreed. Ekambaram found evidence in the structure of the "six-celled" bristles, and in the presence amongst them of short cells, analogous to those in the sensitive hairs of *Aldrovanda*. But the bristles are normally three-celled, and are morphologically equivalent to the three-celled glandular trichomes. The small, so-called, hinge cell does not exist. I have examined, among other species, *U. flexuosa* (Fig. 13–*C*) with which Ekambaram worked. Merl, on the other hand, determined the limiting temperatures, etc., which affect the mechanism getting only a negative answer to the question of irritability. Kruck's argument and supporting evidence has something of challenge in it. It is as follows.

She fixed the trap in position in a bed of agar, so to support it that she could look directly at the door with a suitable objective. Beginning with a set trap, she first made camera lucida drawings of a few cells of the hinge and middle zones (Ansatz, Mittelstück). The bristles were then stimulated by means of a hair, and the hair was then inserted under the free door edge (thus holding the door somewhat open). The same cells as before were now drawn again.

and there appeared distinct differences in the two conditions. During the irritable condition the cells were shorter, broader and had strongly curved walls. The intercellular spaces, especially in the earlier condition, appeared partly very large and filled with air. During the "swallowing" action, the cells elongated, became narrower, and, for the most part, showed no intercellular spaces (presumably the air had been displaced or changed in its shapes). They had become much more translucent in the event that they were still present. After 15 min., the first condition was "almost" completely restored. So much for observation.

The result of this irritable phenomenon Kruck describes thus (Fig. 14). The outer course of cells remains the same, as also the inner in the anticlinal direction. But in the radial direction there is a straightening of the curvature and hence a lengthening of the door. At the same time, the door becomes more lax and gives inwardly, but not so easily outwardly, because here the turgescent cells are. The changes depend on the form of the walls, determined by the thickenings; they are stretched by turgor and become lax by its reduction.

In the hinge zone (Ansatzzone), the cells could be studied as above. In the unstimulated condition they are shorter, the rods (Stifte) closer together, but when stimulated they are longer. In the anticlinal direction the size is not influenced by the broadening of the cells, but the intercellulars and the pectin yield. These cells constitute a hinge which compensates the movement of the middle zone, but at the same time, when at rest, procures a forward pushing of the same. Under stimulation, on the contrary, it draws it back (inwardly ?) somewhat. The zones between the [free] edge and the middle piece are of like structure, but there is effected a broadening through tension just as at the borders of the hinge cells.

Basing her interpretation of the movement of the door on the preceding statements, she proceeds to describe its mechanism. The edge of the door in its unstimulated condition is broad and is drawn back, and, as it were, appressed [against the threshold] by the vaulting of the middle zone. The opening itself is rendered firm by the ring of rigid tissue (threshold). The door and its edge are mutually appressed and rendered rigid by turgescence. The middle zone, equally turgor rigid, is strongly domed in the anticlinal direction and rendered rigid by the studs or rods*. The free edge is difficultly bendable because of its stiff cells. The hinge zone, also stiff, is drawn back in the middle and pushed forward at the edge. In the bristle area, the cells are turgescent, but on account of the rods are broadened but not elevated. The

*In most descriptions much stress is laid on the stiffness of the door, this being attributed to the rods, turgor. Without denying the stiffness, without which it would be no good at all, it seems rather more pertinent to emphasize rather the great flexibility of the door, the function of the rods being to insure the tissue from the crumpling effect of sharp bending which otherwise would cause a collapse of the lateral walls. The rigidity of the door is more than enough to withstand the hydrostatic pressure of the outer medium when equally applied everywhere against a convex surface. Ekambaram (1916) made an interesting rough measure of the resistance of the door to pressure and found that, with the apparatus he used, a pressure of 250 mg. was required to push in the door with a blunt needle. Of course, as he realized, this is not a true measure of the resistance of the door to water pressing equally everywhere.

bristles exhibit a tension of the membranes at the base. What happens on stimulation now follows.

If the bristles are touched the protoplasts contract by giving up water. The tension set up at their bases drags on the plasmodesms and on the walls. The stimulus is transferred to the area about the bristle bases. These cells contract and the door edge draws back "lightning quickly", thereby setting the door slightly open at the edge (Fig. 14–2). The tension of the walls now draws in water. But the middle zone has not yet received the stimulus, and the opening remains yet very narrow.

The stimulus now spreads to the marginal zone, the "Indifferenzzone", and the hinge piece draws back the door edge by the narrowing of its cells. The swallowing (movement) becomes stronger because the middle zone becomes lax and easily bent inward, and at the same time becomes less bowed (domed) and lengthens the door outwardly. The hinge cells become lax and by stretching permit the door to bend inwardly. After the swallowing movement has been completed, the door is pushed against the opening from within (Fig. 14–4). The opening is now closed again and exhaustion of the water by the walls begins. (One would suppose that until the set position is again achieved, which takes "15 minutes", exhaustion of the water could not proceed, since the door is lax.) But the door is not yet irritable, which happens only when the hinge piece is shortened in the middle, the door has bowed itself and the edge stretched forward.

I have been at pains to transcribe the details of Kruck's account, for the rather obvious reason that her view calls for an extraordinarily complex series of behaviors. By way of summary it may be briefly stated that (a) the bristles on receiving a stimulus, contract their protoplasts, expelling water; (b) the stimulus is transferred to the cells of the door immediately surrounding the bases of the bristles, procuring a slight contraction of the door and a release of the door edge, allowing a little water to pass in (under the draught of the trap walls); (c) the stimulus is transferred to the vaulted middle piece and finally to the hinge zone, when the walls of the trap can exert their full draught; (d) after the act of "swallowing" the restitution of the original irritable condition is brought about by a reversal of the whole process, this occupying about 15 min. Kruck postulates changes in the form of the door which accompany the above transference of stimulus, owing to changes in turgor of the cells.

Against the View that the Door is Irritable

In meeting point for point the essential features of this account, we shall first consider if changes in the form and posture of the door occur. To answer this question I have photographed several traps of three species, *U. gibba*, *U. intermedia* and *U. vulgaris*, (a) during the "irritable" state, or, as I would prefer to say, during the set condition; (b) immediately after "stimulation" or tripping, by touching the bristles; (c) after puncturing the wall with a fine needle; and (d) after splitting the same trap along the sagittal plane. These photographs were made very carefully, having regard to the constant orientation of the trap, no lateral pressure being allowed (Plate I; Plate IV–38;

Figs. 7, 13, 15, 16). Diapositives being made, the images were thrown on a screen and tracings made, either of the whole trap, including the door, or merely of the door portion. Only the details which could be clearly seen were traced, namely, so much of the profile of the door as was clearly visible, and the bristles and other outlines, such as the bases of the antennae, the forward edge of the threshold, and certain translucent parts whose relations could not be made out clearly till a sagittal section was examined. I reproduce one such sketch for each species, the details which were clearly visible being duly indicated and distinguishable from the remaining structures. Inspection of the diagrams (Figs. 15, 16) will indicate that the major portion of the outer door surface is visible, as also the bristles, except sometimes quite near their bases.

Specifically, by comparing the photographs as above indicated (Figs. 15, 16), we learn (a) that the convexity of the door surface and its total posture is changed very little after tripping. They are indeed so nearly alike that with the eye alone it is usually impossible to observe any difference. That there is, however, a slight difference in posture is indicated by a slight change in position of the bristles*, some of which may be only apparent owing to slight differences in position of the trap in successive photographs. That some small difference may occur because of the reduced water pressure on the door is only to be expected, but it is difficultly measurable. This appears true also from the fact that when the trap is punctured, thus equalizing the water pressure within and without, the door moves quite measurably farther forward, but the curvature of the door changes but very slightly and chiefly in the region of the hinge (Plate I). If now the derived outlines be compared with a sagittal section and the outlines of the door section and that of the threshold be plotted in, it will be seen that by no stretch of the imagination could we suppose the free door edge to be placed against the inner margin of the threshold, as Kruck has it. It is quite possible to confirm this by making photographs of the door *en face*, both from without and from within, as we have learnt that a total release of the tensions alters the position of the door very little. Taking the front view, from the outside, of a door in an uninjured and set trap, we find that the velum and door edge lie in almost the same optical plane (Plate II–19), such optical condition being impossible if the door edge lay behind the threshold. Cutting the door so that it is not held back by its lateral parts, it swings forward, as every part would, if free (Plate II–14, 15). The above behaviors of the door are easily understandable on the assumption, which I believe to accord with the fact, that the door is simply elastic, and striving by outward movement to take a form from which it is constrained by its attachments. This is not to say, however, that the door is not an irritable system. It conceivably might be and still show the behaviors noted. The evidence, however, is directly opposed to the account given by Kruck and has the advantage of direct and recordable observation.

It will be noted by examination of the diagrams (Figs. 15, 16) that the profile of the trap as a whole changes, as the result of expansion of the lateral walls.

* *The proper position of the door as indicated by that of the bristles was clearly understood by Withycombe (26). See his Fig. 1, p. 402.*

The pressure of water within and without being equalized, after puncturing, the door is moved forward by its own turgor and elasticity, so far as it may be allowed by the velum. There is then a slightly greater curvature at the centre hinge, altering the position of the bristles somewhat. This is very apparent in Fig. 15–*B*, position 4.

Merl argued that if the *Utricularia* trap is an irritable system, changes in the form and distribution of air in the intercellular spaces in the side walls should follow stimulation, but he found none. Kruck used the same argument, directing her attention, as we have seen, specifically to the air spaces in the intercellulars of the door. These occur between the outer and inner courses of cells but are confined usually to the upper part of the frontal triangle *e k k' e'* (Fig. 17–*A*). She claims to have seen such changes within the limits of observability and measurability, following stimulation. She argues therefrom that the cells must have exuded water into the spaces, evidence for irritability.

I have repeated Kruck's experiment, which consisted in observing the door before and after stimulation, the trap being held in suitable position in a bed of agar. It is important to note that in Kruck's experiment, during the observation of the door following stimulation, the door edge was supported by a hair pushed underneath. It is an assumption which may not be justified that this treatment does not disturb the relation of the door curvatures. Instead of a hair I used a very thin wedge of paper which would produce much more distortion of the tissues. Photographs were taken before stimulation, within one minute after stimulation, and in some cases after puncturing the wall with a fine needle. These are reproduced in Figs. 11–13, Plate II. The experiment was several times repeated, and on no occasion could the slightest difference in the distribution of air, or in the shape of the bubbles, be observed. This evidence is perfectly convincing and denies that there is any exudation of water into the intercellular spaces after stimulation, or even after puncturing which procures maximum relaxation of the door. Even this evidence again does not exclude the possibility of irritability, since, if exudation does occur, the amount of contraction of the cell walls, following reduction of volume, would enlarge the intercellular spaces in equal amount. Thus the exuded water might be accommodated without measurable changes in the included air bubbles. We should expect some change, to be sure, but, supposing we should be wrong, we may approach the matter more directly.

Kruck states that the stimulus is received by the bristles, the protoplasts of which contract, the stimulus being thus transferred to the cells about their bases and so on toward the hinge zone. The door surface has been conceded to be insensitive. What now should happen were we to kill the bristles and door with a suitable toxic agent, leaving the walls unaffected? This experiment I did by irrigating the door with strong KI-iodine, holding the set trap in such position that the iodine drained away without touching the walls, or at least without reaching them sufficiently to denature them within the time required for the treatment. I thus irrigated the door until all the trichomes were stained a deep brown, and I have observed in other material that the iodine penetrates very quickly through the cuticle, though some stains (*e.g.*, Ruthenium Red)

do not penetrate at all, and must be allowed to diffuse through the exposed cell surfaces at the torn edge of a dissected-away door. After the trichomes were completely killed and probably also the door cells about the ends of the bristles, if not all (of the extent of the killing we cannot be completely sure), the bristles were touched with a needle and the usual response was obtained. I repeated this experiment a dozen times and recorded one case photographically (Plate II–17, 18). I may remark that it is not quite so easy to spring the trap after such treatment, since the bristles are then not so stiff, having lost their turgor. One must touch them near the bases, avoiding touching the surface of the door (since this is an *ad hoc* experiment). I have already pointed out that the elasticity of the door is a function of turgor and of the physical properties of the cellulose in its conformation peculiar to the door. My experience thus accords with that of Withycombe (26, p. 410) who observed that traps which had lain a half-hour in Bouin's picroformol but which had not been "fired", retaining their set condition, sometimes were discharged by rough handling. He observed that he could not believe the traps to have remained alive (but in spite of the significance of this evidence persisted in regarding the door as an irritable system).

According to Kruck, following a stimulation, the door cannot respond again for a period of 15 min. Merl of course had shown that the set condition can be regained in this period merely because it requires this as a minimum for an exhaustion of water from the interior sufficient to actuate the door on tripping. The following experiment seems to show that Kruck is mistaken. If, after discharging a trap while lying on its side in a film of water, the sides of the trap are carefully compressed with the side of a needle, it is possible to discharge sufficient water to reset the trap. This is not easy, as a very little too much pressure can easily damage its walls. But it can be done. Numerous times I have achieved the discharge of the trap twice (the original and repeat) within a half-minute; sometimes even eight repeats within five minutes, so that it is possible to discharge a trap nine or even more times in about three minutes. If the door is an irritable mechanism, the rest period required is scarcely more than 15 sec. It seems, however, a fair inference that the door is not irritable, but is merely a physical mechanism.

Ekambaram (1916) did this experiment, after some preliminary manipulation consisting of pressing the trap with a pair of pincers. On the release of pressure, though water did not enter the trap, air escaped into it from the intercellular spaces. After this was repeated "twice or thrice", the trap assumed the "hungry" condition, that is, the walls remained collapsed, insuring reduced internal water pressure. Then the bristles were touched and the trap was discharged, this being repeated thrice. Aside from the difficulty of seeing how the intercellular air could have entered the interior unless the cuticle was broken (I have repeated the experiment *ad hoc* but found no entrance of the intercellular air into the trap), Ekambaram's experience coincides with mine, save that I did not find it necessary to undertake the preliminaries. But this author nevertheless holds the view that the mechanism is irritable. In spite of his declaration that "The motor tissues are not confined to the immediate

vicinity of the irritable (*sic*) hairs (that is the bristles) but comprise the collar, valve and the tissue attaching the valve to the orifice of the bladder" he was not able to stimulate the mechanism to action by touching any of these parts, but only when the bristles were "stimulated". It will be recalled that touching the surface of the leaf in *Aldrovanda* (Czaja) and in *Dionaea* (Brown and Sharp) stimulates as well as touching the sensitive hairs, so that, if it is as claimed by Ekambaram and by Kruck, the result is unexpected and unique. But I have already pointed out the common experience of failure to procure action of the door by bending the bristles through no fault in the mechanism, which could be discharged by a heavier touch. The real implication of Ekambaram's experiment escaped him.

It is further pertinent to consider the time relations of the door action. Every observer, from Darwin down, agrees that it is very rapid. On touching the bristles, immediately the sides of the trap bulge, but the door movement is so sudden as to escape the eye. It is very much more rapid than the reaction of the leaf lobes of *Aldrovanda*, though this is among the most rapid movements of the kind known. Kruck concedes that the initial slight withdrawal of the door edge is very rapid ("blitzschnell") but gives us no idea what time the rest of the reaction, namely the spreading of the stimulus to the hinge zone, requires. Moreover, water is said to commence to flow in on the completion of the initial response; this one might argue would nullify the proper effect, which is a rapid movement of a column of water drawn in vigorously by expanding walls. It happens that I have been able to measure the maximum limit of time required by means of moving pictures, and the record shows that the whole movement occupies less than one-sixteenth of a second, the action itself always escaping the camera moving at normal rate, 16 exposures per second. Though the mere rapidity of action is no final argument against the view held by Kruck, it is nevertheless evidence which makes the need of enquiry the more insistent.

A further argument may be drawn from the total movement of the door after discharge, if the evidence in regard to its posture and flexures already advanced may be depended upon. The door, during the period of unstable equilibrium rests with a downward and inward thrust against the forwardly tilted front edge of the middle zone of the pavement epithelium. The release from this position can occur, according to the protagonists of the theory of irritability, only by a change in turgor altering the dimensions of the door one way or the other—shortening it longitudinally (Brocher, Withycombe) or lengthening it by altering the curvatures (Kruck)—which is now the condition that, to be reversed so that the door may regain its irritability, requires a rest period, or rather period of recovery, of at least 15 min. Nevertheless, after discharge the door *immediately falls back into its original form and position.* All except Kruck admit this, against whom the force of this argument is lost unless the premise established by my experiment on posture be accepted. This means either that the recovery of the original turgor conditions must be as rapid as the loss, each phase occupying not more than one-thirty-second of a second, since the whole movement takes less than one-sixteenth of a second, or the door does

not return at once to its original water-tight position and therefore the reduction of water pressure within the trap cannot commence till it does. But Kruck says that the set condition is acquired in 15 min., the changes in the door occupying this period; but a sufficient exhaustion of water by the walls of the trap to reset it also takes a minimum of 15 min. (Merl, Czaja) as I have very many times found myself. Either therefore the exhaustion of the water must go on simultaneously with the recovery of turgor by the door, which is impossible, as Euclid would say, or the exhaustion of water must begin after the door is once again sealed, which would mean 30 min. for the whole process, which is contrary to fact.

An Argument from Comparative Study

During the past three years I have studied material alive or preserved of some 75 species of *Utricularia*. This material I have obtained by the cordial co-operation of colleagues in various parts of the world. Their help I have duly acknowledged in a paper just issued (20). On the basis of the study of their door mechanisms, the species fall into the following groups, presented in tabular form, each group or category being placed under an index species. The numbers are the numbers of my collection.

TABLE I

THE SPECIES OF *Utricularia* (INCL. *Polypompholyx* AS IF *Utricularia*) GROUPED ACCORDING TO THE CATEGORY TO WHICH THEY HAVE BEEN REFERRED. DUPLICATE MATERIAL IS INDICATED BY ITALICS

		Total
Polypompholyx	53, *66*, 100, *104*	2
Hookeri	52, 57, 82, 97, *102*, *103*, 105, *98*, *101*	5
Caerulea	6, 8, 11, 31, 32, 35, *37*, *40*, *55*, *59*, 79, *81*, 85, *91*, 94, *95*	9
Orbiculata	33, *34*, 65, 93	3
Globulariaefolia	16, 45, *62*, *63*, 76, *78*, 96	4
Capensis	1, 4, 5, 48, 84	5
Reniformis	7, 9, 10, 17, *19*, *24*, *25*, 26, 36, 44, 46(?), 50, 56, 58, 61, 64, *75*, 77, 79	15
Subulata	3, 18, *20*, 21, 46(?), 49, *70*, 71, *74*, 89, 90	8
Kirkii	5	1
Vulgaris	2, 15, 23, 28, 29, 30, *38*, 42, *67*, *87*, 88, 92	9
Gibba	13, 22, 27, 39, 41, 51, 54, 60, *72*, *86*	8
Purpurea	14, 47, 68, *69*, 80, *99*	4
Cornuta	12, *43*	1
Lateriflora	83	1
Longiciliata	73	1
		76
	Corr. 1	75

Much of what I might say about these categories has already been said in my Flora paper (20) of this year, I shall therefore indicate only briefly the nature of the argument which arises out of their study. For fuller details see papers Nos. 18–20.

Polypompholyx

The evidence, in the absence of the study of living material, indicates that, in this genus (once regarded as *Utricularia*) the door acts as a valve, in the

sense of Charles Darwin. This view is based on the fact that there is no localized hinge mechanism, either outer hinge or central hinge, and no such localization of areas seen in *U. vulgaris*. The free edge of the door rests on the threshold with a slight overhang on the inside, with no surface resistant to the door edge. There is no extensive velum and no specialized zonation of the very meagre pavement epithelium. But the histology characteristic of the door as a pliable, elastic body is present; rods, infolds, (these do not bulk large in the structure): the latter are irregular, the former slender. In a word the structure is as if the door were all hinge; there being no clear zonation of cells. The outer surface of the door has only low, sessile glands, few in number, and no bristles. The walls of the trap are thick (four courses of cells) and form a triangular structure, as seen in the transverse section, and there seems to be no exhaustion of water therefrom. The trap is, I believe, not capable of setting, in the sense of *U. vulgaris;* or one may say is always set. This judgment is in the absence of the study of the living material and is open to amendment. But the evidence indicates absence of anything but passive movement, yet it has a fundamental histology in common with *Utricularia*, save in those features connected with the nice adjustment of door and threshold to resist water pressure and inleakage.

U. hookeri

This is a type of a number of species all Australasian, in which the door rests on the threshold, somewhat as in Polypompholyx, and there is no surface of resistance of the threshold for the door edge. The door rests on an upturned angle of the threshold but at this point there is a sharp angle of permanent downward (outward) flexure in the door, this being applied to the flexure of the threshold. The threshold has a very broad outer zone, which furnishes part of the velum, and a compact middle zone which is sharply bent transversely into a narrow semicircular (nearly) arc into which the door fits so that the angles of the door are attached to the springs of the arc, while the middle of the door edge overhangs. The only trichomes on the door are low and sessile in two groups, one near the flexure and one on the curving face of the door in the centre of the entrance, which is guarded by an extensive, circular velum. This protects the trap against the inleakage of water which otherwise could take place anywhere easily, as there is everywhere a re-entrant fissure between the threshold and the appressed door. While I have not studied the living material I am assured by my genial correspondents, Mr. A. V. Giblin and Mr. Allan McIntyre, of Hobart, Tasmania, that the walls of the living trap are concave. It seems certain that the trap has a snap action which is procured by a longitudinal fold, started by a slight enforced depression on the surface of the door facing the opening in the circular velum, crossing the permanent transverse flexure from which circumstance the snap action is assured. It is difficult to see, if this interpretation is correct, how such snap action could take place on change in turgor postulated by a theory of irritability, since the vigor of the action can only be proportional to the springiness of the door. It should further be noted that the outer hinge is quite thin while

the thickest part of the door is on either side of the transverse flexure, thus pointing to the need for strength at this point, which would be reduced and the door rendered flabby if the turgor were reduced.

This view is strengthened on examination of the details of structure of the door. This has a very large, relatively inflexible middle piece supported on each side by a more flexible area which can fold, and thus allow the flexure upward of the broad middle piece. The inner portion of the threshold is funnel-shaped beyond the flexure, thus accepting a wide free marginal zone of the door and at the same time allowing its side laps to frill on opening and closing.

The whole aspect of the apparatus lends itself readily when understood to a purely mechanical explanation of opening.

The Groups of the Types Caerulea and Cornuta

The simplest and most striking example of this category is *U. cornuta*, which is devoid of antennae or other appendages. The door curves gently downward, its edge resting against the slightly raised-up tissue of the narrow middle zone of the threshold. There are no bristles, and the only glandular trichomes are a few very low sessile ones on the upper exposed half of the door. I have studied this species in the living condition and find that touching the door is insufficient to cause opening; one has to accomplish a localized thrust of considerable force against the exposed curved upper half of the door enough to dent it slightly, and this initial fold upsets the unstable equilibrium. The absence of any bristles or other trichomes which might be regarded as sensitive make it necessary to assume that the surface of the door is sensitive, if the mechanism is irritable at all; but no experimental evidence has been forthcoming that this is the case. On the contrary, it shows clearly the opposite.

The Group of the Type U. globulariaefolia

This group is very homogeneous. A thick walled trap is provided with heavy antennae very thickly armed with long glandular trichomes forming, together with a similar armature of the ramped stalk, a sort of guiding funnel leading to the central region of a small door without bristles and only a very few sessile glandular trichomes. The aspect of the apparatus aligns it with *U. cornuta*, so far as our present argument is concerned. The same may be said of *U. lateriflora*.

The Type U. purpurea

This is one of the two types of floating submersed forms, *U. vulgaris* being of the other. In the former the ventral profile is almost straight and the threshold lies flat instead of being raised from the wall. The door has a very different form from that of *vulgaris*, having no bristles and no other trichomes excepting only a bunch of long, glandular ones with spherical or fusiform capitals, described by Goebel and by Luetzelburg. They spring from a protuberance arising on the middle line of the door, somewhat above the midpoint. Viewed laterally this appears knob shaped, from above crescentic. The outer hinge is all the region above this, and is thin, the effective hinge

being just above the knob. The lower part of the door is quite stiff, but can buckle longitudinally. Its free edge is provided with a thick forwardly turned weal over which rests the velum. The door edge rests on a narrow dished middle zone of the pavement epithelium. A very effective opponent of the door edge is provided by the permanent swollen cuticles of the inner zone (Plate IV—41, 42). Release of the door edge is caused by any slight rotation of the protuberance, so adjusted that such motion lifts the lower part of the door enough to free it from its opponent. The pressure of water of course does the rest. In springing back into position, the door edge slides over the raised-up cuticles, filled with mucilage, with a little click, thus proving that the dimensions of the door have not changed in discharge.

The sensitivity of the traps varies much, but many are quite as much so as those of *U. vulgaris*, since when being lifted out of the water many are sprung by the water film, and swallow air. When lying in the water, sensitive traps may be sprung by a gentle impact on a few, possibly one, of the glandular hairs which radiate out from the knob. It is however significant that frequently one may gently move the hairs about, pushing them here and there without provoking discharge. It is quite certain that merely touching, or even considerable disturbance of the hairs, is not always effective; but in a case of which the hairs may have been variously manipulated, when they are given a sharp albeit gentle thrust with a flat surfaced needle, without touching the knob, discharge may readily follow. Pressure on the knob from above or below with a needle point can cause discharge. When the touch or thrust is effective the response is momentary as in *U. vulgaris*. My honored colleague of former years, Bashford Dean (10), the only one heretofore to have examined this species in the living condition, was wrong when he denied the occurrence of "spasmodic action".

The behavior of *U. purpurea* quite conclusively denies the possibility of explaining it as an irritable response.

U. reniformis and U. subulata Groups

All essentially alike and obviously similar to the *U. vulgaris* and *gibba* kinds. The most striking species is U. *lloydii* Merl in MS. of which the traps are dimorphic, one having long external glandular trichomes, the other sessile ones. This observation was first made by my friend Dr. Merl. I found that these two forms of the trap, which occur on the same stolon, differ in the presence on the door of a curious bristle (one only) with a hinge mechanism at its base in one sort of trap (that with short trichomes) while this bristle is absent from the other. Both traps catch prey. It is difficult to harmonize these facts with the irritation theory.

U. longiciliata

This is a unique species, the door of which has but one long very slender trichome of the glandular type jutting out from near the middle point of the door (Merl 24). It is very weak and it is doubtful if it is an effective part of the mechanism; and moreover, in the absence of collateral evidence, might be adduced as suggesting irritability. But the rest of the mechanism aligns itself with the *U. caerulea*-like forms.

U. orbiculata

In this curious plant, there are no bristles, but from the door in front of the entrance there are two sausage-like gelatinous masses which arise from peculiar large trichomes, the capital cells of which burst open, the masses of gelatinous matter straightening out to form the nearly cylindrical strands. If the mechanism is an irritable one, the stimulus must be transmitted through the non-living material.

The U. capensis Group

The trap door is longer and narrower relatively, than in *U. vulgaris*, the upper half being bowed and armed with the tuft of glandular trichomes, the lower half being middle piece save at the sides. At its upper limit there is a single large trichome, the capital cell of which is very large and kriss-shaped, and is folded inwardly between the door and the threshold. The way to this narrow cavity is blocked by numerous trichomes arising from the threshold. I have examined living material and have found the trap to act upon touching the upper rounded half of the door which is composed of thin-walled cells and is easily bent in. The equipment is readily understood as a pure mechanism, which experiment indicates it is.

U. kirkii

A unique species, having a door with a shape somewhat as in *U. capensis*, but the middle piece shorter, and possessed on the inner face of two large tubercles separated by a narrow gulley. At their upper limits there are four stiff bristles so adjusted with respect to the threshold and the trichomes forming a funnel-shaped approach thereto, that prey entering the funnel must press on them. The conformation of the door and its relation to the threshold again lend themselves readily to a mechanical explanation as a pure mechanism.

Summary

I have thus briefly indicated that the various mechanisms to be found in a various species of *Utricularia* collectively lend themselves to a purely physical-mechanical explanation. It seems to the writer that when the whole genus (if about one-third of the total number known may be regarded as fairly representative) is surveyed, the inference is that we are here dealing, in the door-threshold apparatus, with purely mechanical "contrivances" all of them conforming to a single general principle, but presenting differences in detail of extraordinary interest. Just as traps, as devised by man, consist of some release mechanism in unstable equilibrium which when tampered with accidentally by a prowling animal nosing the bait snaps and actuates the rest of the trap mechanism, so the door, whether with bristles or without, in some way is possessed of a release mechanism. As such man-made traps have to have a spring or its equivalent, so the traps of *Utricularia* have a source of energy in the out-springly walls, the turgor of whose cells constantly tend to force the walls outward. But, as Brocher first recognized, and as was independently discovered on three other occasions by three different observers, Ekambaram,

Withycombe and Hegner, as Skutch has pointed out, the motive power is usable only because the exhaustion of water from the water-tight trap is accomplished in some way by the walls, that is by their cells, so that the walls collapse. Czaja has called the walls a selective-permeable system, another way of saying the same thing. The trap is therefore far from being "purely passive". This cannot be, as the trap repeatedly sets itself, this being possible only because the door can adjust itself to the entrance and become water-tight. In this the velum is a *sine qua non*, as I have shown experimentally. It acts as a weather stripping, closing the cleft between the door edge and the threshold pavement epithelium and "preventing a draught" (of water). The door itself is complicated and the complications are such as can be understood only if it is postulated that the door has a necessary elasticity and flexibility. To this end the form of the door and of its component cells, and their turgor contribute. A door robbed of its turgor cannot function promptly. Particularly the prompt closing of the door could not fully take place as it does were the turgor of the door reduced, since it closes before the walls are fully, or indeed half, expanded. Even the door therefore is not purely passive, since its effective working depends on its being alive and its cells turgid. But regarded as a mechanism, and assuming its properties, it can be understood in all its details of structure as such. Aside from the general properties of elasticity and flexibility, it is so constructed that it has a definite release or tripping mechanism, the middle piece and its attached bristles (I am speaking now particularly of *U. vulgaris* and its relatives) moving on a special hinge of its own, the central hinge; while the door as a whole has a curved hinge, since its attachment to the trap walls is curved (semi-circular approximately). The structure of the cells in these hinge areas is distinctly correlated with the function of maximum bending and recovery, without which the door could not function at all. The door mechanism is such that it can act as often as there is the occasion of tripping if the walls have performed their duty. The rate at which they can exhaust water is here the limiting factor. About 15 min. after actuation is required for this, but longer is better, as the greater the exhaustion of water, the more pull there is on the inrushing column of water when the door is released. But if we exhaust the trap experimentally, the door will act in normal fashion as long as our patience and skill permit. The answer to the question asked in the title of this discussion is, therefore, in the negative.

Method

A very simple and comfortable method of handling the traps for observation and photography is that of placing them on a bed of cotton wool in water. This allows of easy orientation, holds the trap firmly enough in position, and with a little care the cotton wool does not interfere with the lighting. One spreads over the bottom of a little flat dish a layer of cotton wool and pours on water to cover. The cotton wool should cover the whole bottom, so that it may be anchored by the sides of the vessel. This arragement permits one to irrigate the door without affecting the walls.

For operations on the trap, I have found the small knives used by opthalmologists very useful, especially the Ziegler needle for minute operations on the door, etc., and the von Graefe knife for cutting the whole trap. Operations can be carried on with great nicety under a binocular low power microscope, after practice.

For cutting sagittal and other sections I have found it a practical scheme to hold the trap in position on a slide with a triangle of paper, making the cut with a safety razor blade in a mounting (the "Valet"). A short handle is a distinct advantage.

The best method I have found for repeatedly discharging a trap is to lay it on its side and apply pressure with an opthalmologist's spud on a piece of wet filter paper, which distributes the pressure, under a binocular dissecting microscope.

Appendix

A LIST OF THE SPECIES STUDIED. THE NUMBERS IN TABLE I ARE THOSE IN THIS APPENDIX.

1. *U. capensis*, Spreng.
2. *U. diploglossa*, Web.
3. *U. subulata*, Linn.
4. *U. welwitschii*, Olivier
5. *U. kirkii*, Stapf
6. The same as No. 11
7. *U. reniformis*, A. St. Hil.
8. *U. caerulea*, Auct.
9. *U. longifolia*, Gardn.
10. *U. montana*, Jacq.
11. *U. gibbsiae*, Stapf
12. *U. cornuta*, Michx.
13. *U. pallens*, A. St. Hil. & Girard
14. *aff. cucullata*
15. *U. oligosperma*, A. St. Hil.
16. *U. globulariaefolia*, Mart.
17. *U. lundii*, A. DC.
18. *U. nervosa*, G. Weber.
19. *U. reniformis*, A. St. Hil.
20. *U. subulata*, Linn.
21. *U. resupinata*, B. D. Greene
22. *U. gibba*, Linn.
23. *U. vulgaris*, Linn.
24. *U. reniformis*, A. St. Hil.
25. *U. reniformis*, A. St. Hil.
26. *U. endresii*, Reichb.
27. *aff. gibba*
28. *U. intermedia*, Hayne
28a. *U. intermedia*, Hayne
29. *U. stellaris*, Linn.
30. *U. flexuosa*, Vahl.
31. *U. albo-caerulea*, Dalz.
32. *U. affinis*, Wight.
33. *U. caerulea*, Linn.
34. *U. orbiculata*, Wall.
35. *U. uliginosa*, Vahl.
36. *U. dusenii*, Sylvén.
37. *U. caerulea*, Auct.
38. *U. intermedia*, Hayne
39. *aff. gibba*
40. *U. caerulea*, Linn.
41. *U. pumila*, Walt.
42. *U. mixta*
43. *aff. cornuta*
44. *U. gluckii*, Luetz.
45. *U. amethystina*, Salzm.
46. *U. nana*, A. St. Hil. & Girard
47. *U. elephas*, Luetzelburg
48. *U. puberula*, Benj.
49. *U. pusilla*, Vahl.
50. *U. geminiloba*, Benj.
51. *U. exoleta*, R. Br.
52. *U. volubilis*, R. Br.
53. *Polypompholyx multifida*, F. Muell.
54. *U. neottioides*, A. St. Hil. & Girard
55. *aff. caerulea*
56. *U. dusenii*, Sylvén.
57. *U. menziesii*, R. Br.
58. *U. saudadensis*, Merl.
59. *aff. caerulea*
60. *U. herzogii*, Luetzelburg
61. *U. campbelliana*, Oliver
62. *U. amethystina*, Salzm.
63. *U. globulariaefolia*, Mart.
64. *U. nelumbifolia*, Gardn.
65. *U. orbiculata*, Wall.
66. *Polypompholyx multifida*, F. Muell.
67. *U. vulgaris*, Linn.
68. *U. purpurea*, Walt.
69. *U. cucullata*, St. Hil.
70. *aff. subulata*
71. *U. triloba* (prob.), Benj.
72. *U. neottioides*, A. St. Hil. & Girard
73. *U. longiciliata*, A. DC.
74. *U. subulata*, Linn. (or prob. *U. nervosa*, G. Weber)
75. *U. dusenii*, Sylvén.
76. *U. tridentata* (prob.), Sylvén.
77. *U. lloydii*, Merl in MS.
78. *U. globulariaefolia*, Mart.
79. *U. flaccida*, A. DC.
80. *U. myriocista*, A. St. Hil. & Girard
81. *U. uliginosa*, Vahl.
82. *U. dichotoma*, Labill.
83. *U. lateriflora*, R. Br.
84. *U. albina*, Ride
85. *U. bifida*, Linn.
86. *U. exoleta*, R. Br.
87. *U. flexuosa*, Vahl.
88. *U. geminiscapa*, Benj.

89. *U. subulata forma cleistogama* (Gray) Fern.
90. *U. resupinata*, B. D. Greene
91. *aff. caerulea*
92. *U. flexuosa*, Vahl.
93. *U. striatula*, Sm. vel. *orbiculata*
94. *U. reticulata*, Sm.
95. *U. bifida*, Linn.
96. *U. modesta*, A. DC.
97. *U. monanthos*, Hook.
98. *U. violacea*, R. Br.
99. *U. purpurea*, Walt.
100. *Polypompholyx tenella*, Lehm.
101. *U. dichotoma*, Labill.
102. *U. menziesii*, R. Br.
103. *U. hookeri*, Lehm.
104. *Polypompholyx multifida*
105. *U. violacea*

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EXPLANATION OF PLATES

PLATE I

FIGS. 1–6. *Utricularia gibba. Silhouettes of trap to show door posture. 1, Living trap in the set condition; 2, same after discharge; 3, same after puncturing the wall; 4, same, a portion of the cheek cut away to show the relations of the door more clearly; 5, sagittal section, living; 6, the same, fixed with iodine and cleared with glycerol. See Fig. 15–A.*

FIGS. 7–10. *U. intermedia, silhouettes of trap to show the door posture. 7, in set condition; 8, after discharge; 9, after puncturing; 10, sagittal section, living. See Fig. 16.*

PLATE II

FIGS. 11–13. *U. intermedia. Front views of the door in the same trap to show the intercellular air before discharge (Fig. 11), after discharge (Fig. 12) and when the door edge is held up by a small paper wedge. Note that the pattern formed by the air remains unchanged.*

FIG. 14. *U. intermedia. Position taken by the door when freed by cutting away just in front of the threshold and lying in water.*

FIG. 15. *Position taken by a narrow strip of the median part of the door when freed by cutting and lying in water.*

FIG. 16. *Same after plasmolysis in 0.5N potassium nitrate.*

FIG. 17. *U. intermedia. Trap before discharge, after all the trichomes on the door have been killed with iodine; and*

FIG. 18. *same after discharge.*

FIG. 19. *Front of door of U. gibba to show the velum lying against the surface of the door just above the edge. Note that the top edge of the velum (indicated by the pointer) is caught under the short trichomes arising from the surface of the door just below the level of the lower pair of bristles. Living.*

FIG. 20. *Median section through the threshold of U. intermedia, showing the outer (to the left of pointers), middle (between pointers) and inner zone (right of pointers) of the pavement epithelium; and showing also the velum attached to the cells of the outer zone. Remains of the cuticle walls project from between the cells of the inner zone.*

FIG. 21. *U. intermedia. Door exposing the inner surface showing the character of the epidermal cells of the inner course. See Fig. 17–A for guidance in recognizing the various areas.*

FIG. 22. *U. vulgaris. Threshold flattened out to show the various zones of the pavement epithelium, the middle zone, between the paired white pointers, ending at the black pointer. The outer zone above, inner zone below, separated by single white pointer at the left. Compare with Plate V–47.*

PLATE III

FIGS. 23–30. *A series of sections of the threshold at different points, the positions of which can be determined by looking at Fig. 13–A (p. 397). Fig. 23, section at the extreme end of the threshold; the door seen crossing it; Fig. 24, a little lower (between 1 and 2, Fig. 13–A) the velum can be seen at the left; Fig. 25, at point 2; Fig. 26, between points 2 and 3 but nearer point 2; Fig. 26, about half way between points 2 and 3; Fig. 27, at point 3; Fig. 28, between points 3 and 4, but nearer 3; Figs. 29 and 30, at or near the middle point. This series is to show the change in form of the threshold from point to point.*

FIG. 31. *U. vulgaris. A very young, scarcely functional trap which shows well the structure of the velum. The cuticular caps of the capital cells of the middle zone form a membrane attached to the bladdery cuticles formed by the capital cells of the outer zone. Inner zone not shown.*

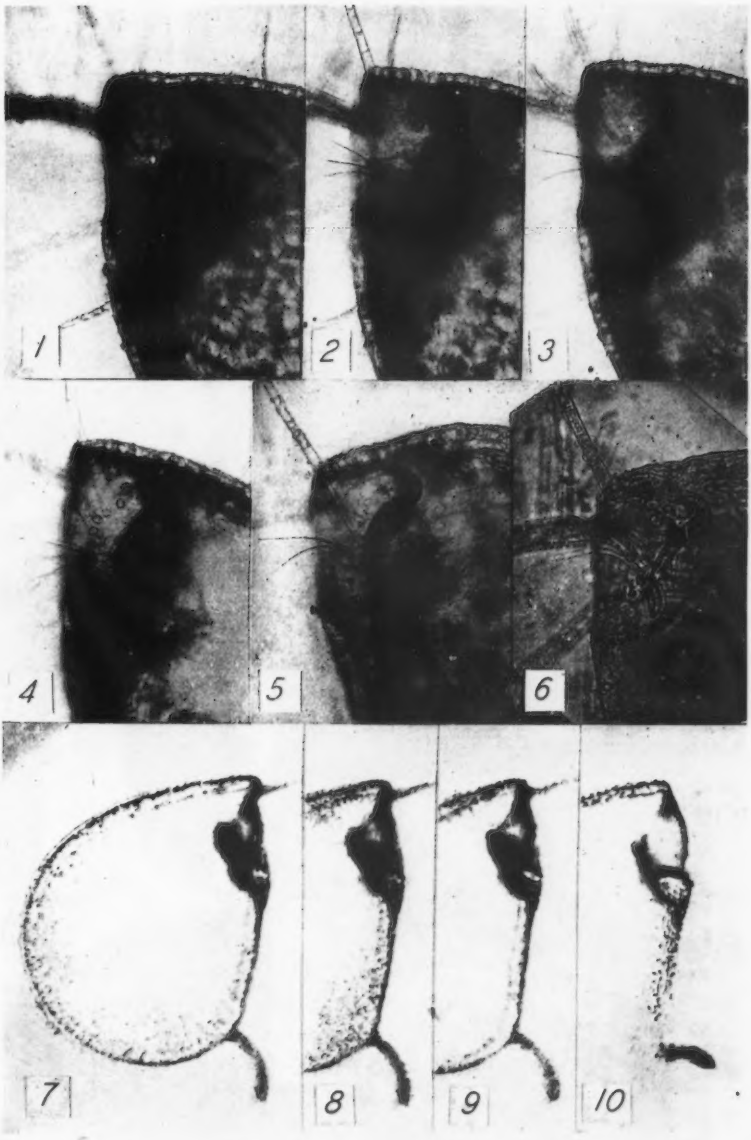
FIG. 32. *Part of inner surface of door between a, b, and c, Fig. 17–A, the preparation having lain flattened out in water for some time. The cells are distorted and quite out of their natural condition, which can be seen in the corresponding area in Fig. 33.*

FIG. 33. *Note however that the ends of entire cells near the cut edge of the door are even here distended, having no normal living cells to oppose them. These "spring"-like cell walls are not to be found in the uninjured door. Compare Fig. 22. The completely normal form of these cells may be seen in Plate IV–35, except, again, at the cut margins, well shown at the upper left, U. intermedia.*

FIG. 34. *The cell of the inner course of the door along a narrow zone (the edge zone) showing the numerous rods. These cells are very like those of the outer hinge.*

PLATE IV

FIG. 35. *Inner epidermis of the door of U. vulgaris. For photographic purposes advantage was taken of the presence of anthocyanin in the cells to get a "negative" picture in which the cell walls are for the most part dark. The upper hinge shown is fragmentary, being torn in dissection, but these cells show in the lower left hand corner as the hinge zone merges into the edge zone, and in Fig. 37. Note that the "circular lines" are not seen except where the middle zone cells are tilted toward the eye, and are elsewhere replaced by grooves, the infolds of the outer walls (cf. Fig. 39 of this plate). The bulbous swellings of the middle piece (indicated by the pointer) lie where the two paired groups of larger cells are seen between the central hinge and the lower half of the middle piece. The small cells of the central hinge have relatively very large rods. The details of this region are better seen in Fig. 36.*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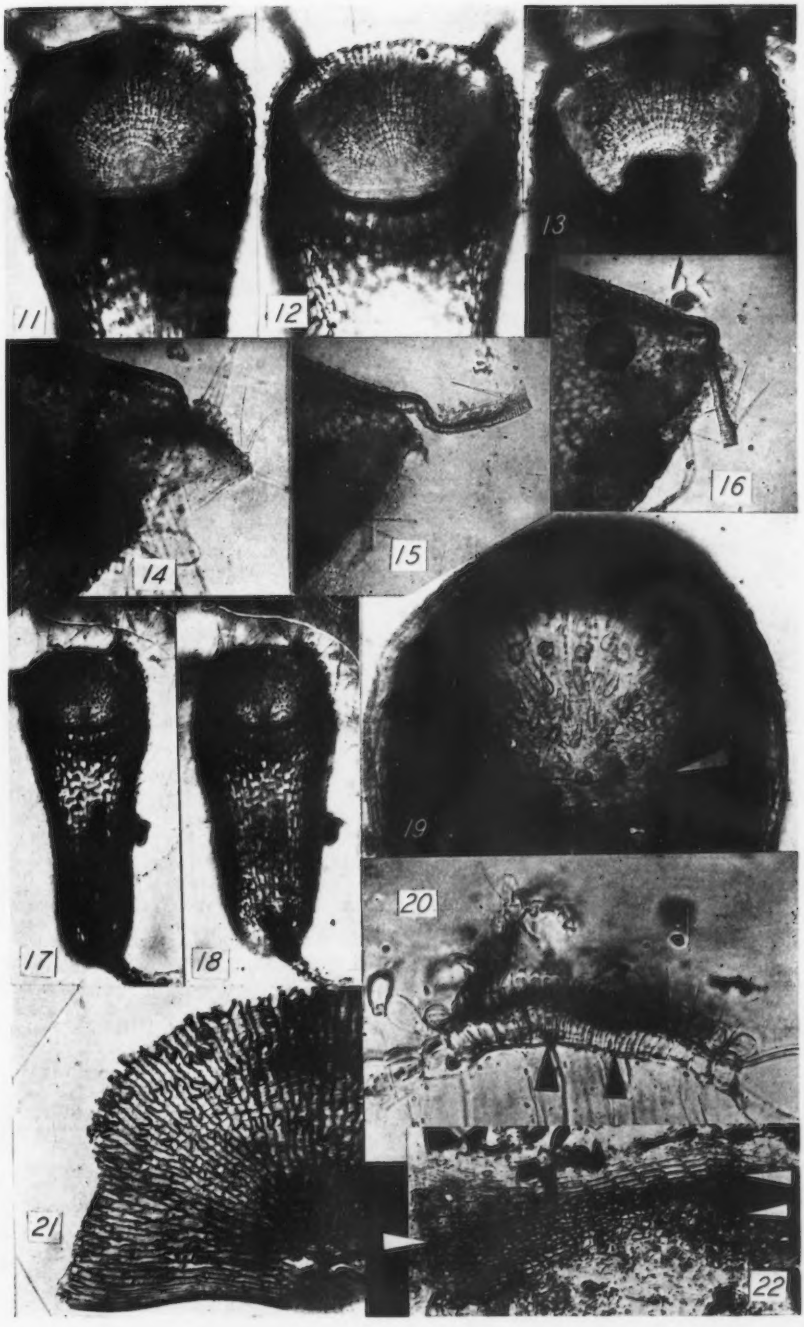
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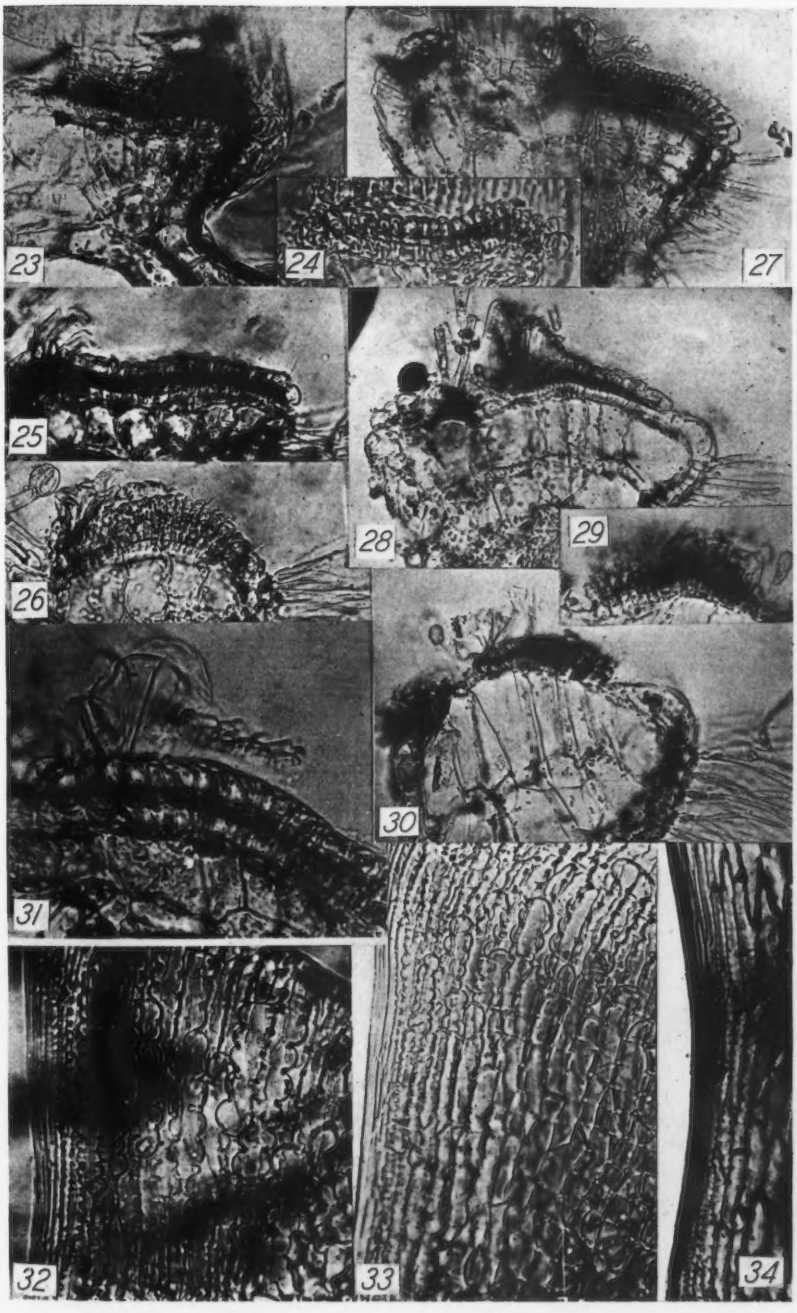
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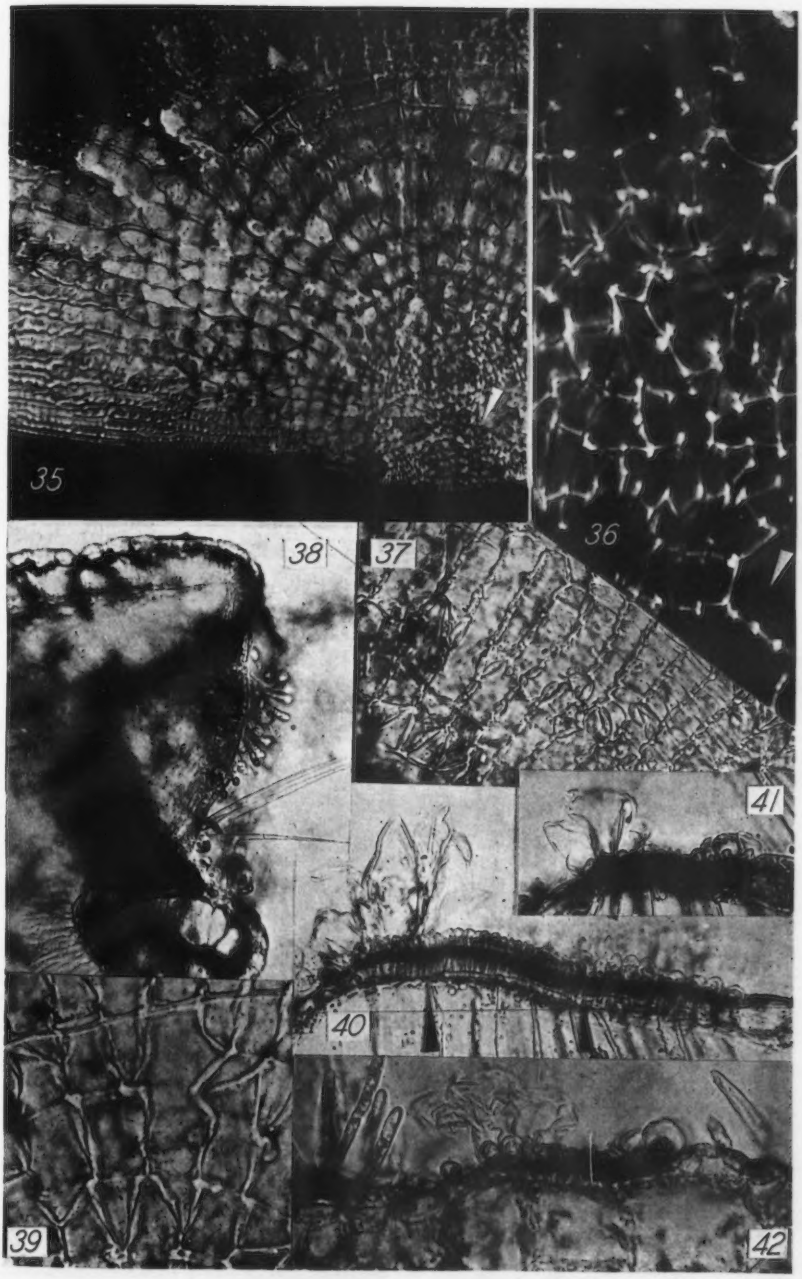
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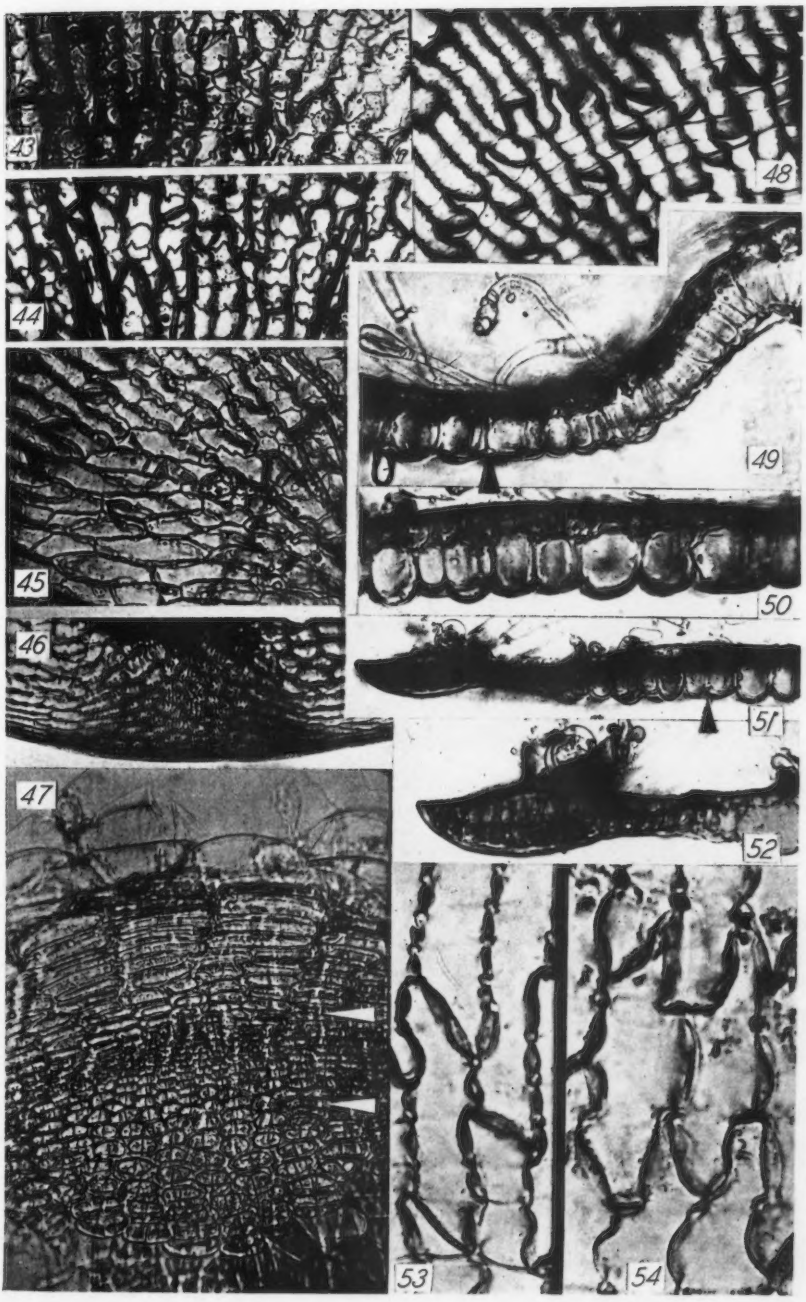
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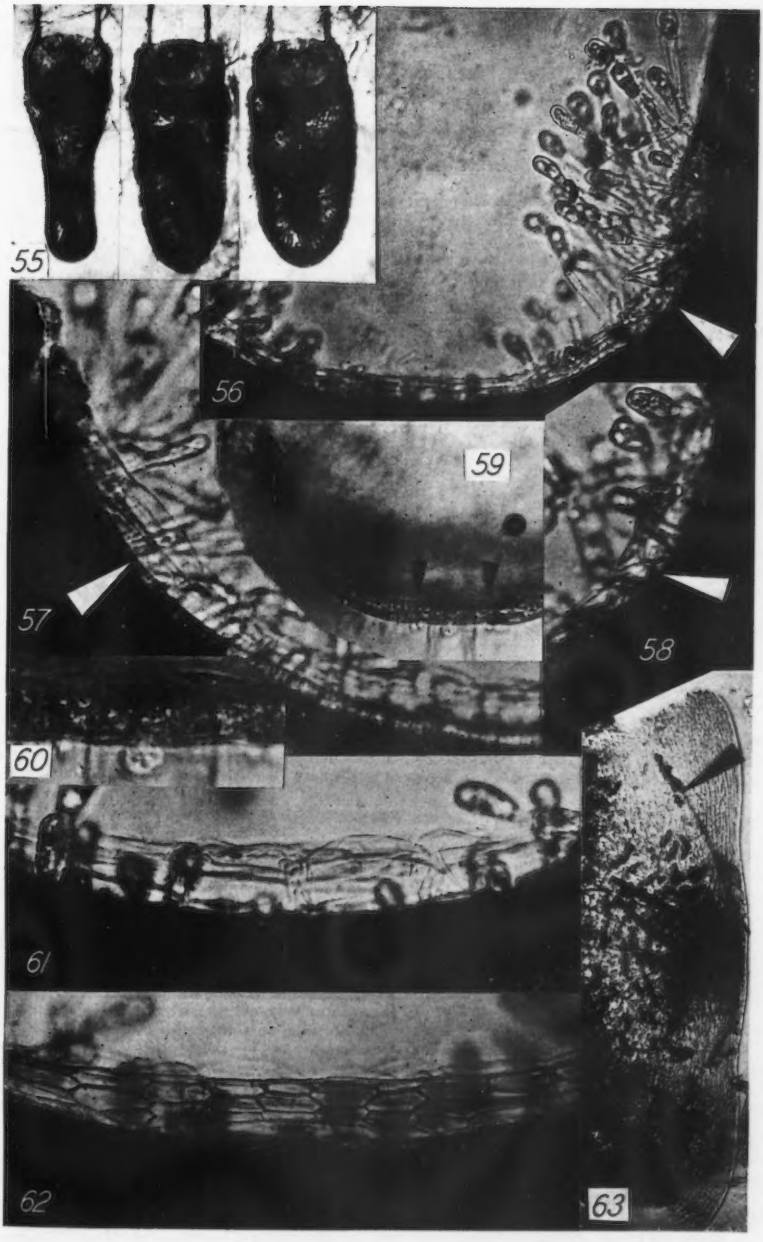
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FIG. 36. *The zig-zag infolds of the outer cell walls can be seen, becoming longitudinal and roughly parallel as the central hinge merges into the middle piece. This figure is the right half (approx.) of the central hinge of another preparation. The pointers indicate corresponding cells, for orientation purposes. On the upper left of Fig. 35, distorted cells with the "spring"-shaped walls.*

FIG. 37. *A portion of the outer hinge zone. The walls with more numerous rods. Compare with Plate V–48, in which the circular lines can be seen, only faintly indicated in this figure (Fig. 37).*

FIG. 38. *Sagittal section of the living door region of U. intermedia. The door is not quite in the normal closed position. Some details are of course obscured by shadows cast by the threshold and the insertion of the upper hinge. Compare with Fig. 13–A, which was made with this preparation as a basis.*

FIG. 39. *A small portion of the inner epidermis of the middle zone of the door, to show one "circular line" and the only faint double shadows made elsewhere. For optical interpretation see Fig. 23–A.*

FIG. 40. *Median section of the threshold of U. vulgaris. Middle zone between the pointers, outer on the left, inner on the right. The slight groove affording the resting place for the middle piece of the door can be seen just in front of the ample velum.*

FIG. 41. *Threshold of U. aff. cucullata (Hoene, 3070, Brazil). This is like that of U. purpurea. Velum in front of slightly dished narrow threshold, with a bolster of inflated cuticular membranes of the inner zone of pavement epithelium.*

FIG. 42. *The same but from another preparation.*

PLATE V

FIGS. 43–46. *Outer epidermis of the door of U. intermedia.*

FIG. 43. *Outer hinge zone (cf. Fig. 21–B).*

FIG. 44. *Middle zone. The shadowy walls of the inner course of cells can be seen (cf. Fig. 21–C).*

FIG. 45. *From tension zone along line l l', Fig. 17–A, where there is change from transverse to radial orientation of the cells (cf. Fig. 21–A).*

FIG. 46. *The middle piece (cf. Figs. 23–B and 24–B).*

FIG. 47. *En face view of the middle portion of the threshold of U. vulgaris, in which the character of the capital cells of the outer, middle and inner threshold zone can be seen, together with the velum attached to the outer zone cells. Only the outer cells, the capitals, of the three-celled glandular trichomes can be seen.*

FIG. 48. *Inner epidermis of outer hinge of door, to show the "circular lines". For optical explanation see Figs. 23–A and 24–A.*

FIGS. 49–52. *Sagittal section of the door, U. intermedia.*

FIG. 49. *Upper hinge zone, ending at the pointer.*

FIG. 50. *Middle zone; the thin inner infolded walls of the outer course can best be seen in this section.*

FIG. 51. *Middle zone merging into the central hinge and this into the middle piece. The infold of an outer wall of the inner epidermis, without support of rods, can be seen clearly at the pointer.*

FIG. 52. *The middle piece, showing the rods to be broad at the outer walls and smaller at their inner ends.*

FIG. 53. *Inner door epidermis, outer hinge zone, to show the numerous regularly cylindrical rods. Stained with Ruthenium red.*

FIG. 54. *Inner door epidermis, middle zone, showing the irregularly, more widely spaced rods, and no transverse "bars". Ruthenium red.*

PLATE VI

FIG. 55. *A living trap of U. intermedia, before and after discharge (resp. left hand and middle) and after the velum had been cut on one side (right). The result of this operation is seen in the next figure.*

FIG. 56. *The velum of the trap seen in Fig. 55, showing the cut, indicated by the pointer. Compare with the uninjured velum on the other side seen in*

FIG. 57. *Uninjured velum, on the right side, facing the door.*

FIG. 58. *Another experiment. Only the cut velum is shown.*

FIG. 59. *A door, living, removed and viewed edgewise to show the thickenings on each side of the middle point (between the pointers). Seen enlarged in*

FIG. 60. *Front of door facing downwards. The two thickenings of the middle piece, projecting inwardly.*

FIG. 61. *View of the middle reach of the velum of U. intermedia from the front. The membranes are derived from the bladdery cuticles of the outer zone of the threshold.*

FIG. 62. *The same as seen from the inside of the trap. The membrane here seen is derived from he cuticles of the middle zone of the threshold.*

FIG. 63. *Part of the door of a trap killed by some hours' immersion in Ruthenium red. The stain collected in the chink where the velum edge lay and consequently traced the line of the velum edge across the door. The pointer indicates the line. U. vulgaris.*

A MATHEMATICAL THEORY OF THE GROWTH OF POPULATIONS OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM*, DUV.

II. THE DISTRIBUTION BY AGES IN THE EARLY STAGES OF POPULATION GROWTH[1]

By John Stanley[2]

Abstract

In a previous paper (2) a mathematical theory of the growth of populations of the flour beetle, *T. confusum* Duv., was developed, and a function $\theta(T)$ descriptive of the rate at which eggs are arriving at hatching age at a time, T, was described in a cursory manner. This function has now been written $\theta(T,\gamma)$ giving the number of eggs of age γ at time T, where $t_0=0<T<t_3$, t_3 being the time at which the first egg hatches.

$\theta(T,\gamma)$ has been developed, and its characteristics have been described by means of a study of its partial derivatives, whence it has been possible to plot a frequency-distribution surface for the numbers of eggs of various ages during this early stage of population growth.

Introduction

In a previous paper (2), the author developed a mathematical theory descriptive of the growth of populations of the flour beetle, *T. confusum*, Duv., the populations being grown in small environments each consisting of 32 gm. of whole wheat flour.

The adequate notation set up in that paper will be used unchanged in this present investigation, and need not be repeated in tabular form.

During the discussion of the appearance of larvae in the populations (2), a function, $\theta(T)$, descriptive of the rate at which eggs are arriving at hatching age at a time T was introduced. Its form, however, was not developed.

It is now proposed to develop an extended form of $\theta(T)$ such that, at any time $t_2=t_0=0\leq T\leq t_3$, *i.e.*, from the commencement of the growth of the population to the moment of hatching of the first egg, it shall be possible to determine the number of eggs of ages from γ to $\gamma+d\gamma$ present in the flour mass.

Discussion

It was shown, (2, p. 657) that

$$\theta(t_3)=R\epsilon N_{11}-\int_{t_2}^{t_3}\frac{\theta(t)C'_{11\cdot 2}}{N_2}\,dt, \tag{1}$$

where $C'_{11\cdot 2}=\dfrac{HN_2}{cN_2+d}$, N_2 equals the number of eggs at a time T.

We shall now replace $\theta(t_3)$ by $\theta(T,\gamma)$ where T is any instant of time $t_2\leq T\leq t_3$, and γ is the age of any egg. Then $\theta(T,\gamma)$ defines a family of frequency distributions in γ, T being thought of as a parameter determining which curve of the family shall be considered at any time.

[1] *Manuscript received August 22, 1932.*
Contribution from the National Research Laboratories, Ottawa, Canada.

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Then where $\alpha_1 = R\epsilon N_{11}$ (see 2, p. 649)

$$\theta(T,\gamma) = \alpha_1 - \int_{T-\gamma}^{T} \frac{H\theta(t,\gamma)}{cN_2+d}\, dt \tag{2}$$

Equation 2 is an example of *Volterra's Equation* (1, p. 13) in the case of which, if certain necessary and sufficient conditions are satisfied, one and only one solution can be obtained, and that by the term-by-term integration of an absolutely and uniformly convergent series developed by the method of "successive substitutions."

As Equation 2 can be shown to satisfy these conditions, we proceed to its solution by the above-mentioned method.

Substituting in Equation 2 the value of $\theta(T,\gamma)$ as determined from the equation itself,

$$\theta(T,\gamma) = \alpha_1 - H\int_{T-\gamma}^{T} \frac{\alpha_1 - \int_{T-\gamma}^{t} \frac{H\theta(t,\gamma)}{cN_2+d}\, dt}{cN_2+d}\, dt, \tag{3}$$

from which

$$\theta(T,\gamma) = \alpha_1 - H\alpha_1 \int_{T-\gamma}^{T} \frac{dt}{cN_2+d} + H^2 \int_{T-\gamma}^{T} \frac{dt}{cN_2+d} \int_{T-\gamma}^{t} \frac{\theta(t,\gamma)}{cN_2+d}\, dt. \tag{4}$$

By successive repetitions of this process there is obtained the absolutely and uniformly convergent series:

$$\theta(T,\gamma) = \alpha_1 - H\alpha_1 \int_{T-\gamma}^{T} \frac{dt}{cN_2+d} + H^2\alpha_1 \int_{T-\gamma}^{T} \frac{dt}{cN_2+d} \int_{T-\gamma}^{t} \frac{dt}{cN_2+d}$$
$$- H^3\alpha_1 \int_{T-\gamma}^{T} \frac{dt}{cN_2+d} \int_{T-\gamma}^{t} \frac{dt}{cN_2+d} \int_{T-\gamma}^{t} \frac{dt}{cN_2+d} + \ldots\ldots \text{ etc.} \tag{5}$$

It was shown (2, Eq. 27) that

$$\frac{dN_2}{dt} = \frac{aN_2+b}{cN_2+d}, \tag{6}$$

which, as $a << 0$ (2, p. 648), may be written

$$\frac{dN_2}{dt} = \frac{b-|a|N_2}{cN_2+d}. \tag{7}$$

From Equation 7,

$$dt = \frac{(cN_2+d)}{(b-|a|N_2)}\, dN_2\ . \tag{8}$$

Substituting the value of dt from Equation 7 in Equation 5, and denoting by $F(T)$ the value of N_2 at time T,

$$\theta(T,\gamma) = \alpha_1 - H\alpha_1 \int_{F(T-\gamma)}^{F(T)} \frac{dN_2}{b-|a|N_2} + H^2\alpha_1 \int_{F(T-\gamma)}^{F(T)} \frac{dN_2}{b-|a|N_2} \int_{F(T-\gamma)}^{F(t)=N_2} \frac{dN_2}{b-|a|N_2} - \ldots \text{ etc.} \tag{9}$$

Integrating term by term, as it is legitimate to do, by reason of the fact that Equation 2 satisfied the required necessary and sufficient conditions, and writing

$$\Phi = -\frac{H}{|a|}\left[\log_e\left\{b-|a|F(T)\right\} - \log_e\left\{b-|a|F(T-\gamma)\right\}\right], \tag{10}$$

it is found that

$$\theta(T,\gamma) = c_1+\alpha_1\left[1-\Phi+\frac{\Phi^2}{2!}-\frac{\Phi^3}{3!}+\frac{\Phi^4}{4!} \quad - \ldots \text{ etc.}, \right. \tag{11}$$

where c_1 is the sum of all the constants of integration.*

The series in Φ converges to the value $e^{-\Phi}$ for all values of Φ, $-\infty < \Phi < +\infty$, hence

$$\theta(T,\gamma) = c_1+\alpha_1 e^{-\Phi} = c_1+\alpha_1\left[\frac{b-|a|F(T-\gamma)}{b-|a|F(T)}\right]^{-\frac{H}{|a|}}. \tag{12}$$

When $T=0$, $\gamma=0$, as no eggs were laid prior to $T=t_2=0$. Also, when $T=0$, $N_2=F(0)=0$, and $\theta(T,0)=R\epsilon N_{11}$ (see 2, p. 649).

Hence
$$\theta(T,0) = R\epsilon N_{11} = c_1+\alpha_1\left(\frac{b}{b}\right)^{-\frac{H}{|a|}} \tag{13}$$

so that $c_1=0$.

It must be remembered that $a<<0$. Hence to avoid confusion, we write

$$\theta(T,\gamma) = R\epsilon N_{11}\left[\frac{b-|a|F(T)}{b-|a|F(T-\gamma)}\right]^{\frac{H}{|a|}} \tag{14}$$

As $\theta(T,\gamma)$ determines the number of eggs of age γ at a time T, $t_2 \leq T \leq t_3$, clearly, at any time T, the sum of the eggs of all ages from $\gamma=0$ to $\gamma=T$ must be equal to $F(T)$, the total number of eggs at time T, *i.e.*,

$$\int_{\gamma=0}^{\gamma=T} R\epsilon N_{11}\left[\frac{b-|a|F(t)}{b-|a|F(t-\gamma)}\right]^{\frac{H}{|a|}} dt = F(T) \tag{15}$$

We proceed to show that this is so. Set $t-\gamma=Z$, whence $-d\gamma=dZ$. When $\gamma=0$, $T-\gamma=T=Z$; when $\gamma=T$, $T-\gamma=0=Z$, and the integral becomes, reversing the limits to eliminate the minus sign of dZ,

$$R\epsilon N_{11}\left[b-|a|F(T)\right]\int_0^T \frac{dZ}{[b-|a|F(Z)]^{\frac{H}{|a|}}}. \tag{16}$$

But $F(Z)=N_2$ at the point Z, whence, setting $R\epsilon N_{11}\left[b-|a|F(T)\right]^{\frac{H}{|a|}} = \beta$,

we obtain $$\beta\int_0^T \frac{dZ}{[b-|a|N_2]^{\frac{H}{|a|}}}. \tag{17}$$

That this sum is finite follows from the absolute and uniform convergence of the series of Equation 9.

From (2, Eq. 27), $dZ = \frac{(cN_2+d)}{(b-|a|N_2)}\, dN_2$.

When $Z=0$, $N_2=0$, and when $Z=T$, $N_2=F(T)$ whence the integral becomes

$$\beta \int_0^{F(T)} \frac{(cN_2+d)}{(b-|a|N_2)^{\frac{H}{|a|}+1}}\, dN_2. \tag{18}$$

Set $b-|a|N_2=y$, then $cN_2+d=\frac{cy-(bc+|a|d)}{-|a|}$, and $dN_2=\frac{y}{-|a|}$.

When $N_2=0$, $y=b$, and when $N_2=F(T)$, $y=b-|a|F(T)$, so that the integral becomes

$$\frac{\beta}{(-|a|)^2} \int_b^{b-|a|F(T)} \left[cy^{-\frac{H}{|a|}} - (bc+|a|d)y^{\left(-\frac{H}{|a|}-1\right)} \right] dy. \tag{19}$$

This is immediately integrable, and becomes

$$\frac{R\epsilon N_{11}}{(-|a|)^2}\left[b-|a|F(T)\right]^{\frac{H}{|a|}} \cdot \left[\frac{-|a|cy}{H-|a|}^{\left(-\frac{H}{|a|}+1\right)} + \frac{(bc+|a|d)\,(|a|)y^{-\frac{H}{|a|}}}{H} \right]_b^{b-|a|F(T)}. \tag{20}$$

From (2, p. 648), $H-|a|=R\epsilon N_{11}c$, whence on substituting the limits and simplifying somewhat, we obtain

$$-\frac{[b-|a|F(T)]}{|a|} + \frac{R\epsilon N_{11}(bc+|a|d)}{H(|a|)} + \frac{R\epsilon N_{11}[b-|a|F(T)]}{|a|}^{\frac{H}{|a|}} \left[\frac{b^{\left(-\frac{H}{|a|}+1\right)}}{R\epsilon N_{11}} - \frac{(bc+|a|d)b^{-\frac{H}{|a|}}}{H} \right]. \tag{21}$$

Consider the two terms within the square brackets on the right. From (2, p. 648), $b=R\epsilon N_{11}d$, whence

$$\frac{b}{H}^{-\frac{H}{|a|}} = \frac{b^{\left(-\frac{H}{|a|}+1\right)}}{HR\epsilon N_{11}d},$$

so that the last term of (21) is

$$\frac{[b-|a|F(T)]}{|a|}^{\frac{H}{|a|}} \left[\frac{Hdb^{\left(-\frac{H}{|a|}+1\right)} - (bc+|a|d)b^{\left(-\frac{H}{|a|}+1\right)}}{Hd} \right]$$

$$= \frac{[b-|a|F(T)]}{|a|}^{\frac{H}{|a|}} \cdot \frac{b^{\left(-\frac{H}{|a|}+1\right)}(Hd-bc-|a|d)}{Hd}. \tag{22}$$

But, from (2, p. 648) $Hd-bc-|a|d=|a|d+R\epsilon N_{11}cd-|a|d-R\epsilon N_{11}cd=0$, whence the last term on the right of formula (21) is zero, and there remains only

$$-\frac{[b-|a|F(T)]}{|a|} + \frac{R\epsilon N_{11}(bc+|a|d)}{H(|a|)},$$

which, from (2, p. 648),

$$= F(T) - \frac{b}{|a|} + \frac{R\epsilon N_{11}(bc+|a|d)}{H(|a|)} = F(T) - \frac{1}{H(|a|)}\left[bH - R\epsilon N_{11}(bc+|a|d) \right]$$

$$= F(T) - \frac{1}{H(|a|)}\left[b(|a|+R\epsilon N_{11}c) - R\epsilon N_{11}bc - R\epsilon N_{11}(|a|)d \right] \tag{23}$$

$$= F(T) - \frac{1}{H(|a|)}\left[(|a|)b - R\epsilon N_{11}|a|d\right] = F(T) \quad \text{(Q.E.D.)} \tag{24}$$

Characteristics of the Frequency-distribution Surface Defined by $\theta(T,\gamma)$

As the operations of finding the various derivatives of $\theta(T,\gamma)$ involve only the ordinary rules of partial differentiation, the intermediate steps may be omitted. It is thus seen that:

$$\lim_{T\to 0} = \lim_{F(T)\to 0} \theta(T,\gamma) = R\epsilon N_{11} > 0, \tag{25}$$

$$\lim_{\gamma\to 0} = \lim_{(T-\gamma)\to T} \theta(T,\gamma) = R\epsilon N_{11} > 0, \tag{26}$$

$$\lim_{T\to \infty}{}^{*} = \lim_{F(T)\to \xi} \theta(T,\gamma) = 0, \tag{27}$$

$$\lim_{\gamma\to T} = \lim_{(T-\gamma)\to 0} \theta(T,\gamma) = R\epsilon N_{11}\left[1 - \frac{|a|}{b}F(T)\right]^{\frac{H}{|a|}} > 0. \tag{28}$$

$$\frac{\partial\theta(T,\gamma)}{\partial T} = -R\epsilon N_{11}H\left[\frac{b-|a|F(T)}{b-|a|F(T-\gamma)}\right]^{\frac{H}{|a|}}\cdot\left[\frac{1}{cF(T)+d} - \frac{1}{cF(T-\gamma)+d}\right] > 0, \tag{29}$$

$$\lim_{T\to 0} = \lim_{F(T)\to 0} \frac{\partial\theta}{\partial T} = 0, \tag{30}$$

$$\lim_{\gamma\to 0} = \lim_{(T-\gamma)\to T} \frac{\partial\theta}{\partial t} = 0, \tag{31}$$

and, as from (2, p. 648) $\xi = \frac{b}{|a|}$,

$$\lim_{T\to \infty} = \lim_{F(T)\to \xi} \frac{\partial\theta}{\partial T} = 0, \tag{32}$$

$$\lim_{\gamma\to T} = \lim_{(T-\gamma)\to 0} \frac{\partial\theta}{\partial T} = -R\epsilon N_{11}H\left[1 - \frac{|a|}{b}F(T)\right]\left[\frac{1}{cF(T)+d} - \frac{1}{d}\right] > 0. \tag{33}$$

$$\frac{\partial\theta(T,\gamma)}{\partial\gamma} = -\frac{R\epsilon N_{11}H}{[cF(T-\gamma)+d]}\left[\frac{b-|a|F(T)}{b-|a|F(T-\gamma)}\right]^{\frac{H}{|a|}} < 0, \tag{34}$$

$$\lim_{T\to 0} = \lim_{F(T)\to 0} \frac{\partial\theta}{\partial\gamma} = -\frac{R\epsilon N_{11}H}{d} < 0, \tag{35}$$

$$\lim_{\gamma\to 0} = \lim_{(T-\gamma)\to T} \frac{\partial\theta}{\partial\gamma} = -\frac{R\epsilon N_{11}H}{cF(T)+d} < 0, \tag{36}$$

$$\lim_{T\to \infty} = \lim_{F(T)\to \xi} \frac{\partial\theta}{\partial\gamma} = 0, \tag{37}$$

$$\lim_{\gamma\to T} = \lim_{(T-\gamma)\to 0} \frac{\partial\theta}{\partial\gamma} = -\frac{R\epsilon N_{11}H}{cF(T)+d}\left[1 - \frac{|a|}{b}F(T)\right]^{\frac{H}{|a|}} < 0. \tag{38}$$

* *In an actual case, these formulations hold only if $T < t_h$, but it is of interest to suppose hatching to be in some way indefinitely deferred, and to examine the consequences.*

$$\frac{\partial^2\theta(T,\gamma)}{\partial T\partial\gamma} = \frac{\partial^2\theta(T,\gamma)}{\partial\gamma\partial T}$$

$$= \frac{ReN_{11}H}{[cF(T-\gamma)+d]}\left[\frac{b-|a|F(T)}{b-|a|F(T-\gamma)}\right]^{\frac{H}{|a|}}\left[\frac{c\{b-|a|F(T-\gamma)\}}{\{cF(T-\gamma)+d\}^2} - H\left\{\frac{1}{cF(T)+d} - \frac{1}{cF(T-\gamma)+d}\right\}\right] > 0. \quad (39)$$

$$\lim_{T\to 0} = \lim_{F(T)\to 0} \frac{\partial^2\theta}{\partial T\partial\gamma} = \frac{ReN_{11}Hbc}{d^3} = \frac{(ReN_{11})^2Hc}{d^2} > 0, \quad (40)$$

$$\lim_{\gamma\to 0} = \lim_{(T-\gamma)\to T} \frac{\partial^2\theta}{\partial T\partial\gamma} = \frac{ReN_{11}Hc[b-|a|F(T)]}{[cF(T)+d]^3} > 0, \quad (41)$$

$$\lim_{T\to\infty} = \lim_{F(T)\to\xi} \frac{\partial^2\theta}{\partial T\partial\gamma} = 0, \quad (42)$$

$$\lim_{\gamma\to T} = \lim_{(T-\gamma)\to 0} \frac{\partial^2\theta}{\partial T\partial\gamma} = \frac{ReN_{11}H[bc-H(d^2-d)]}{d^3[cF(T)+d]}\left[1-\frac{|a|}{b}F(T)\right]^{\frac{H}{|a|}} > 0. \quad (42a)$$

$$\frac{\partial^2\theta(T,\gamma)}{\partial T^2} = -ReN_{11}H\left(\frac{b-|a|F(T)}{b-|a|F(T-\gamma)}\right)^{\frac{H}{|a|}}H\left[\frac{1}{cF(T)+d} - \frac{1}{cF(T-\gamma)+d}\right] +$$

$$ReN_{11}H\left(\frac{b-|a|F(T)}{b-|a|F(T-\gamma)}\right)^{\frac{H}{|a|}}\left[c\frac{\left\{\frac{b-|a|F(T-\gamma)}{cF(T-\gamma)+d}\right\}}{\{cF(T-\gamma)+d\}^2} - c\frac{\left\{\frac{b-|a|F(T)}{cF(T)+d}\right\}}{\{cF(T)+d\}^2}\right] < 0 \quad (43)$$

That $\frac{\partial^2\theta(T,\gamma)}{\partial T^2} < 0$ may be shown as follows:

Let $cF(T)+d=x$, $cF(T-\gamma)=y$, then $x>y$, and the discriminant, D, of $\frac{\partial^2\theta(T,\gamma)}{\partial T^2}$ is the quantity within the square brackets, *i.e.*,

$$D = -H\left(\frac{1}{x}-\frac{1}{y}\right)^2 + \frac{cF'(T-\gamma)}{y^2}^{*} - \frac{cF'(T)}{x^2} \quad (44)$$

$$D = -\frac{H}{x^2} + \frac{2H}{xy} - \frac{H}{y^2} + \frac{cF'(T-\gamma)}{y^2} - \frac{cF'(T)}{x^2}$$

As $x>y$, $x^2>y^2$; also $F'(T-\gamma)>F'(T)$ from (2, Eq. 30), where $0<F(T)<\xi$

Whence $\frac{cF'(T-\gamma)}{y^2} - \frac{cF'(T)}{x^2} > 0$ (46)

also $$\frac{H}{xy} - \frac{H}{x^2} > 0\ ,\ \frac{H}{xy} - \frac{H}{y^2} < 0, \quad (47)$$

whence $$\frac{cF'(T-\gamma)}{y^2} - \frac{cF'(T)}{x^2} + \frac{H}{xy} - \frac{H}{x^2} > 0. \quad (48)$$

Now $-\frac{H}{x^2} > -\frac{H}{y^2}$, whence, from Equation 40,

$$\frac{cF'(T-\gamma)}{y^2} - \frac{cF'(T)}{x^2} - \frac{H}{x^2} > -\frac{H}{x^2} > -\frac{H}{y^2}\ , \quad (49)$$

*By $F'(T-\gamma)$ is meant the first partial derivative $\frac{\partial F(T-\gamma)}{\partial T}$

and as obviously $\frac{H}{xy} = \frac{H}{xy}$, it is seen that $D>0$, whence $\frac{\partial^2\theta(T,\gamma)}{\partial T^2} < 0$.

Further, it can be shown that:

$$\lim_{T\to 0} = \lim_{F(T)\to 0} \quad \frac{\partial^2\theta(T,\gamma)}{\partial T^2} = 0, \tag{50}$$

$$\lim_{\gamma\to 0} = \lim_{(T-\gamma)\to T} \quad \frac{\partial^2\theta}{\partial T^2} = 0, \tag{51}$$

$$\lim_{T\to\infty} = \lim_{F(T)\to\xi} \quad \frac{\partial^2\theta}{\partial T^2} = 0, \tag{52}$$

$$\lim_{\gamma\to T} = \lim_{(T-\gamma)\to 0} \quad \frac{\partial^2\theta}{\partial T^2} = 0. \tag{53}$$

$$\frac{\partial^2\theta(T,\gamma)}{\partial\gamma^2} = R\epsilon N_{11}H\left[\frac{b-|a|F(T)}{b-|a|F(T-\gamma)}\right]^{\frac{H}{|a|}}\cdot\left[H\left\{\frac{1}{cF(T-\gamma)+d}\right\}^2 + \frac{c\left\{b-|a|F(T-\gamma)\right\}}{\left\{cF(T-\gamma)+d\right\}^3}\right] > 0, \tag{54}$$

$$\lim_{T\to 0} = \lim_{F(T)\to 0} \quad \frac{\partial^2\theta}{\partial\gamma^2} = R\epsilon N_{11}H\left(\frac{Hd+bc}{d^3}\right) > 0, \tag{55}$$

$$\lim_{\gamma\to 0} = \lim_{(T-\gamma)\to T} \quad \frac{\partial^2\theta}{\partial\gamma^2} = \frac{R\epsilon N_{11}H[H+cF'(T)]}{[cF(T)+d]^2} > 0, \tag{56}$$

$$\lim_{T\to\infty} = \lim_{F(T)\to\xi} \quad \frac{\partial^2\theta}{\partial\gamma^2} = 0, \tag{57}$$

$$\lim_{\gamma\to T} = \lim_{(T-\gamma)\to 0} \quad \frac{\partial^2\theta}{\partial\gamma^2} = \frac{R\epsilon N_{11}H(Hd+bc)}{d^3}\left[1-\frac{|a|}{b}F(T)\right] > 0. \tag{58}$$

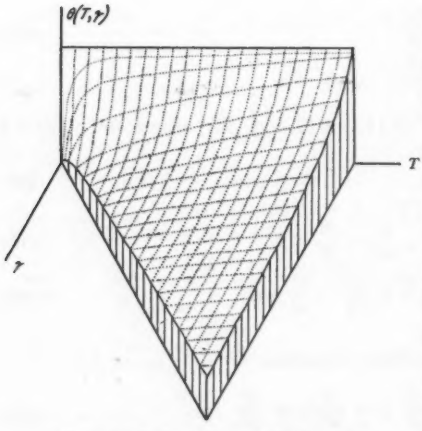

FIG. 1 *General form of the frequency-distribution surface* $\theta(T,\gamma)$.

From the information obtained in Equations (25) to (58) it is possible to plot the surface $\theta(T,\gamma)$ in character as shown in Fig. 1.

Thus, under the conditions set forth, the number of eggs of age γ at a time T is a function of T and γ, Equation (14). Moreover, as T increases from 0 to $+\infty$, the number of eggs of any age γ, $(0<\gamma<\infty)$ increases (Equation 29) at an ever decreasing rate (Equation 43). The number of eggs of age zero, *i.e.*, those just laid, remains however at $R\epsilon N_{11}$ (Equation 26), while the number of the oldest eggs, *i.e.*, those of age T. increases according to Equation (28),

Also, at any time the older eggs are less numerous than the younger, (Equation 34), though this difference, for equal differences in age, becomes less and less apparent as T increases (Equation 39).

Moreover, as N_2 approaches ξ, (at an infinite time,) the numbers of eggs of infinite age approaches zero, these having been entirely eliminated by prolonged exposure to eating (Equation 28)*.

It is of interest to examine $\frac{H}{|a|}$

It can be shown from (2, p. 648) that

$$\frac{H}{|a|} = \frac{E_{11}}{E_{11} - R\epsilon W_2 A_2}$$

and as E_{11} is the maintenance rate of ingestion of a mature adult (2, p. 647) and $R\epsilon W_2 A_2$ the rate of expenditure of assimilable nutrient material in the form of eggs, $\frac{H}{|a|}$ is the reciprocal of the "efficiency" of a mature adult as a growing locomotory machine.

The biological substantiation of the above described formulations would of course necessitate the determination of the ages of numerous eggs. This may be accomplished by determining the additional time (at the same temperature as that at which the original experiment was performed) necessary for the hatching of the particular eggs under consideration. The probable errors, (see 2, Table I) are less than .1 day for temperatures between 27° and 32° C., and only .7 day at temperatures as low as 17° C.

The writer hopes to carry out this biological substantiation in the near future.

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*This may be seen by placing $F(T) = N_2 = \xi = \frac{b}{|a|}$ in Equation 28, when the term in the square brackets becomes zero.

DURATION OF META-STABLE STATES, NEON[1]

By J. M. Anderson

Abstract

The rate of decay of absorption of six neon lines λ 6402, λ 6334, λ 6266, λ 6163, λ 6143 and λ 5945 has been measured for a series of pressures at room temperature and at the temperature of liquid air. Curves showing the variation of half-life with pressure have been plotted for each line at both temperatures. It is found that the function $\log \frac{I}{I_o} = \frac{D_1 - D_2}{\gamma}$ is exponential in the time, and that the rate of decay is markedly different at the same pressure and temperature for different lines ending in the same meta-stable state.

In previous papers the author has dealt with a simple theory of meta-stable atomic states, especially those of the inert gases (1) and has given experimental determination of the duration of the absorption shown by λ 7635 in argon, $^3P_2(p^5p) - {}^3P_2(p^5s)$, of which the lower state is meta-stable (2). This work has now been extended to the neon spectrum and the decay curves for the absorption as shown by several lines have been determined for a series of pressures both at room temperature and at the temperature of liquid air.

Apparatus

The apparatus used is the same as that described in the previous paper (2) with only such changes as were necessitated by repair work. The electrical circuit is shown in (2, Fig. 4). G_1 and G_2 are two 750 watt two-pole 50 cycle A.C. generators using 220 volts D.C. input and giving 110 volts A.C. output. These are coupled on the same shaft by the variable coupling C which is graduated in four hundred equal divisions.

The output of G_1 excites transformer T_1 whose output passes through the rectifying valves V, the resistance CR_1, the discharge tubes D_1 and D_2 and the synchronous switch B_1 which is fastened to the shaft of the exciting generator. The output of T_1 is shunted through the condenser C_1 which takes up the surges caused by the breaking of the circuit by B_1. The essential feature of this circuit is that the switch B_1 must make a positive metal-to-metal contact in order to assure sufficiently low excitation to allow the lines emitted to be absorbed by an undisturbed gas. Flashes of excitation in the tube D_2 are found to last for about 10^{-4} sec. and are not followed by stray discharge due to the action of the switch B. This switch also serves to assure that the flash will occur at precisely the same time in each cycle.

The output of G_2 excites transformer T_2 whose output passes through the resistance of CR_2, the meter $M.A.$, the discharge tubes D_3 and D_4 and the synchronous switch B_2. A condenser is also shunted across this transformer. The essential feature is that the switch B_2 depends on the breaking down of an air gap to complete the circuit. This renders the excitation in tube D_4 rather high, but ensures flashes of an extremely short duration ($<10^{-5}$ sec.). High

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Contribution from the Physical Laboratory, University of Toronto, Toronto, Canada.

excitation is fortunately not a matter of concern in this circuit as the absorption by D_4 takes place after the discharge has been cut off so that the atoms are in zero field. Only one-half the cycle is used from both T_1 and T_2 in order to allow a longer time between successive excitations of the tubes.

The experimental method consists in producing excited atoms in tube D_4 by means of a discharge, and observing the amount of absorption suffered by various lines in the flash of light emitted by D_2 a known time after the discharge in D_4 has ceased. This known time is varied by means of the coupling C fixing the relative phase of the armatures and switches simultaneously. The tubes D_1 and D_3 are observed by means of their reflections in the mirror M set at an angle to the shaft of the generators. Thus, the character of the discharge given by the two circuits and the time difference between the two flashes given by them can be definitely checked.

The absorption tube used is shown in (2, Fig. 6). It is necessarily enclosed in a vacuum flask for low temperature work. Tungsten electrodes were used which were heated white hot at frequent intervals to ensure that all impurities were driven off. The emission tube consisted of a Geissler tube bent back and forth several times to increase the luminous area without increasing the depth of emitting gas. It was placed directly below the absorption tube. Light passed up through the absorption tube was reflected at the mirror placed in the metal cover, out to a high light-power spectrograph. By means of a system of mirrors, light from the same emission tube impressed a comparison spectrum on the plate at the same time as the exposure was being taken. This precaution was found to be absolutely essential to accurate estimation of the absorption.

Experimental

The neon used was partly purified by passage over charcoal immersed in liquid air. It was then admitted to a bulb in which a heavy discharge was passed between a potassium pool and an auxiliary electrode. This process produced a gas of very high purity in a very short time. The gas used in the emission tube was only roughly purified, as traces of impurity in the emission tube can have no effect on the life of the absorption shown by the gas in the absorption tube, and changes in intensity due to slight variations of purity were automatically cancelled out by the method of taking the comparison spectrum as described above. Ilford Special Rapid Panchromatic plates were used, developed in glycin. Density curves were made on the Moll self-recording microphotometer, densities being calculated from the formula $D_2-D_1=\log\frac{h_0-d_1}{h_0-d_2}$, in which D_1 and D_2 are the photographic densities produced

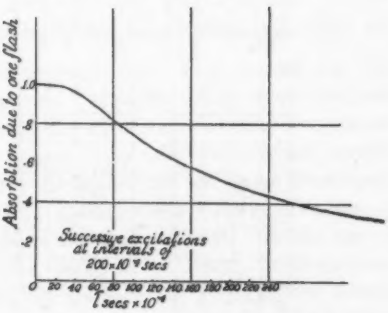

FIG. 1. *Effect of successive excitations.*

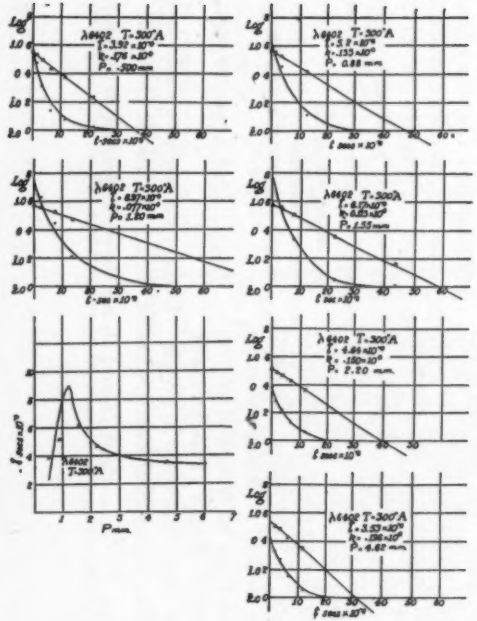

FIG. 2. *Decay curves of λ 6402; T = 300° A.*

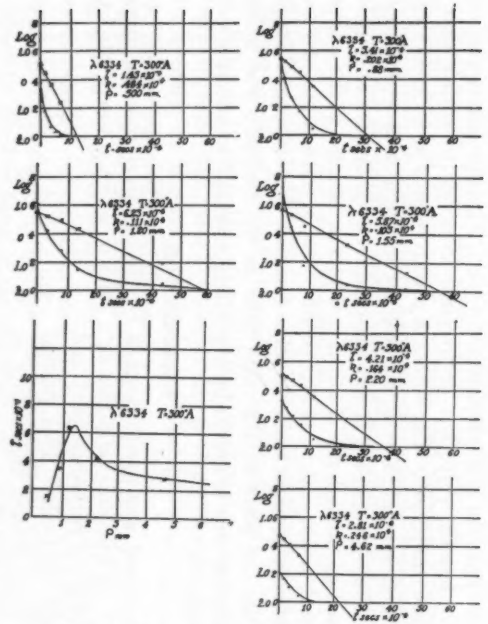

FIG. 3. *Decay curves of λ 6334; T = 300° A.*

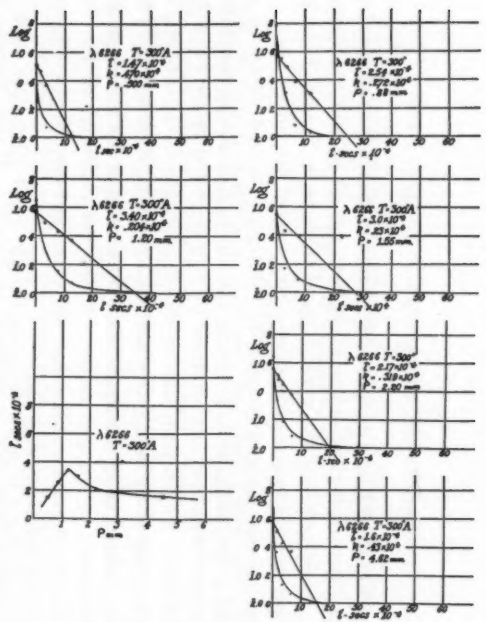

FIG. 4. *Decay curves of λ 6266; T = 300° A.*

on the plate by the absorbed and comparison spectra, h_0 the total microphotometer deflection from clear plate to saturation density, and d_1 and d_2 the deflections of the microphotometer produced by the lines of the comparison and absorbed spectra. The law $\frac{D_1-D_2}{\gamma}=\log\frac{I_1}{I_2}$ in which I_1 and I_2 are the actual intensities of light in the spectra, and γ is a constant pertaining to the plate, was assumed as the connection between I and D. This was found to be true within the limits of experimental error for the intensities and wave-lengths studied. Pressures were measured by means of a McLeod gauge.

The current through the absorption tube was kept constant by means of the meter directly in series with the tube. The current to produce the same absorption was necessarily smaller for liquid air temperatures than for room temperatures, as in these cases the excited atoms have not completely disappeared in the 1/50 sec. which elapses between successive excitations of the absorption tube. The absorption is then represented by a series:

$$A=C_e^{-kt}+C_e^{-k(t+T)}+C_e^{-k(t+2T)}=Ce^{-kt}\cdot\frac{1}{1-e^{-kT}}=C_1e^{-kt}$$

It will be noted that the absorption still decays exponentially with time but with a different and larger constant C. The effect on the absorption is shown in Fig. 1, in which it is seen that the effect is only appreciable when τ, the half-life, is comparable with T the period of excitation. It was found that within the limits for which absorption was experimentally observable the half-life was, as expected, independent of the exciting current. Owing to the peculiar wave form of the current (flashes of the order of 10^{-5} sec. once every $\frac{1}{50}$ sec.), D.C. calibration means little. However, the meter reading used corresponded to D.C. readings of approximately 100 micro-amperes.

Results

The results obtained are shown in the Figs. 2 to 13, in which the experimental values of $D_2-D_1=\log\frac{h_0-d_1}{h_0-d_2}$ are shown in heavy black dots (the units are arbitrary and not strictly comparable throughout). The crosses are the logarithms of these values on the scale shown. Half-life values were obtained from the slope of the straight lines through these crosses. It may be pointed out that the curves drawn through the values of D_1-D_2 are intended merely to indicate the general course of the absorption and are not to be considered as giving an accurate value as, owing to the scale necessary for publication, it was found impossible to draw the steeper curves accurately. By the use of more convenient scales of plotting, half-life values as read from the absorption curves agreed with those obtained from the slope of the straight line within the error of either. It will be seen that the plotting of log (D_1-D_2) against time gives an excellent straight line especially at the liquid air temperatures, a result fully as good as could be expected from absorption measurements which in themselves contain an error of the order of 5%. The better agreement at the low temperature is due largely to the minimizing of the effect of the error in

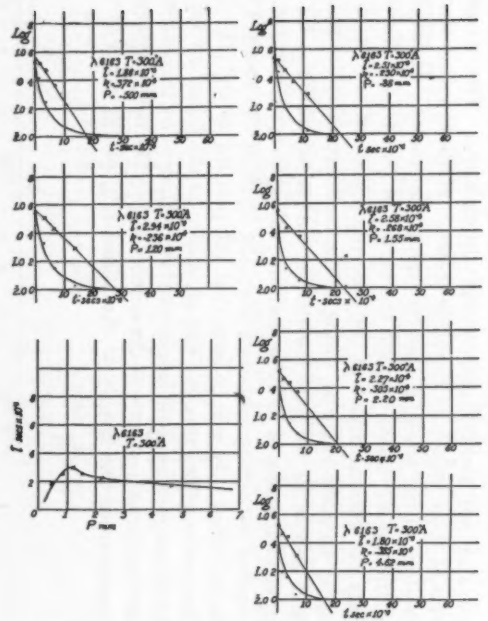

FIG. 5. *Decay curves of λ 6163; T = 300° A.*

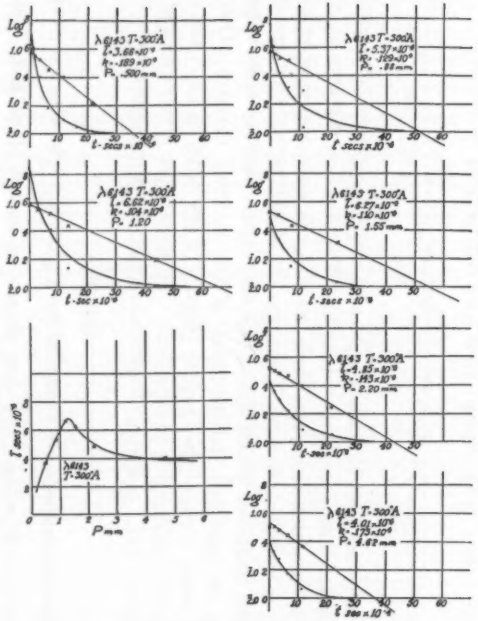

FIG. 6. *Decay curves of λ 6143; T = 300° A.*

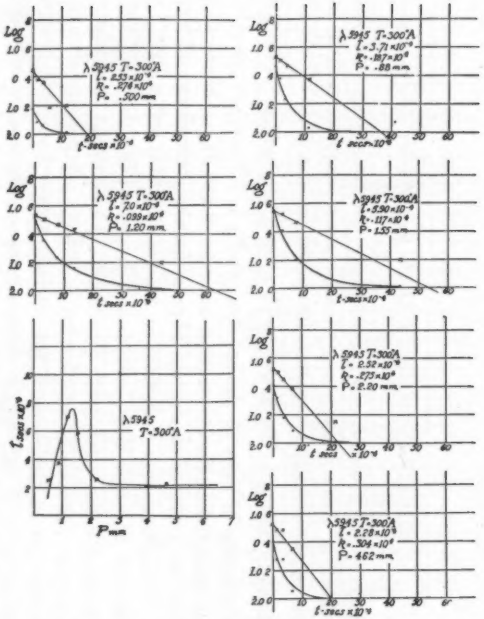

FIG. 7. *Decay curves of λ 5945; T = 300° A.*

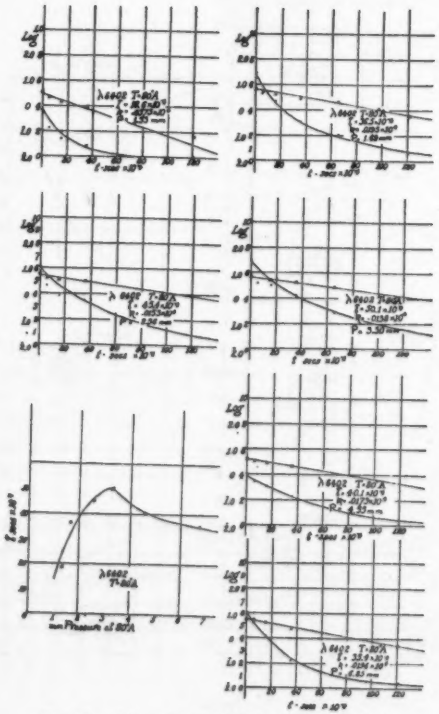

FIG. 8. *Decay curves of λ 6402; T = 80° A.*

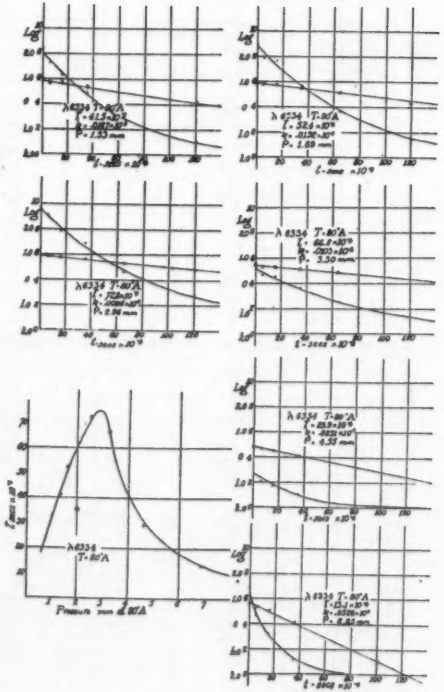

FIG. 9. *Decay curves of λ 6334; T = 80° A.*

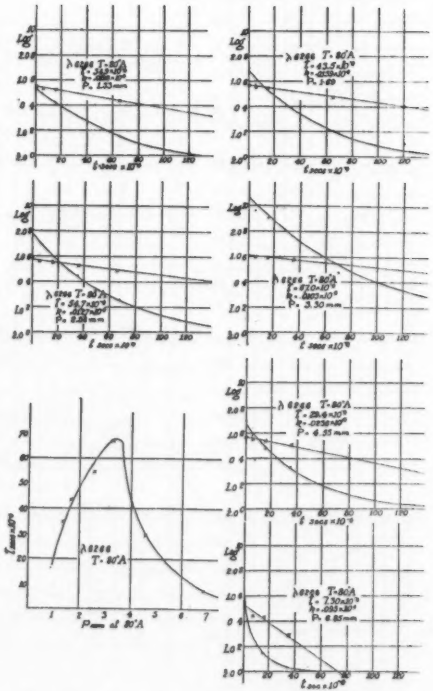

FIG. 10. *Decay curves of λ 6266; T = 80° A.*

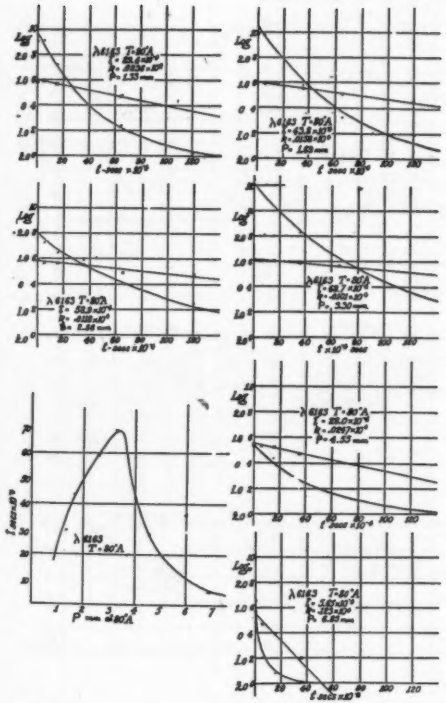

FIG. 11. *Decay curves of λ 6163; T=80° A.*

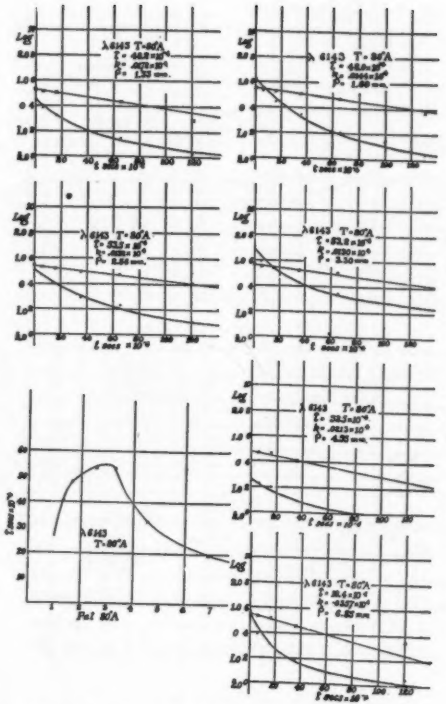

FIG. 12. *Decay curves of λ 6143; T=80° A.*

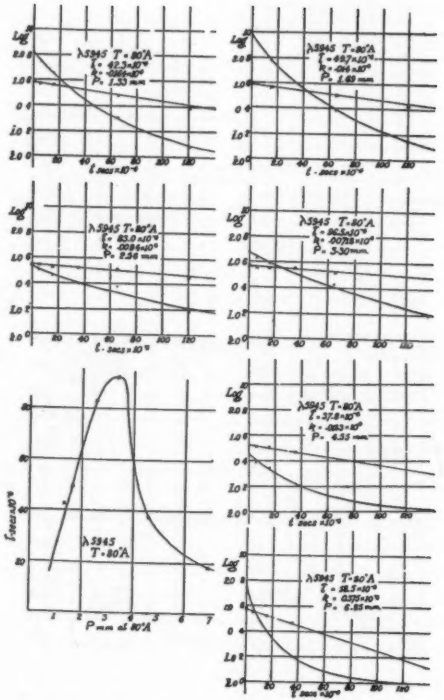

FIG. 13. *Decay curves of λ 5945; T=80° A.*

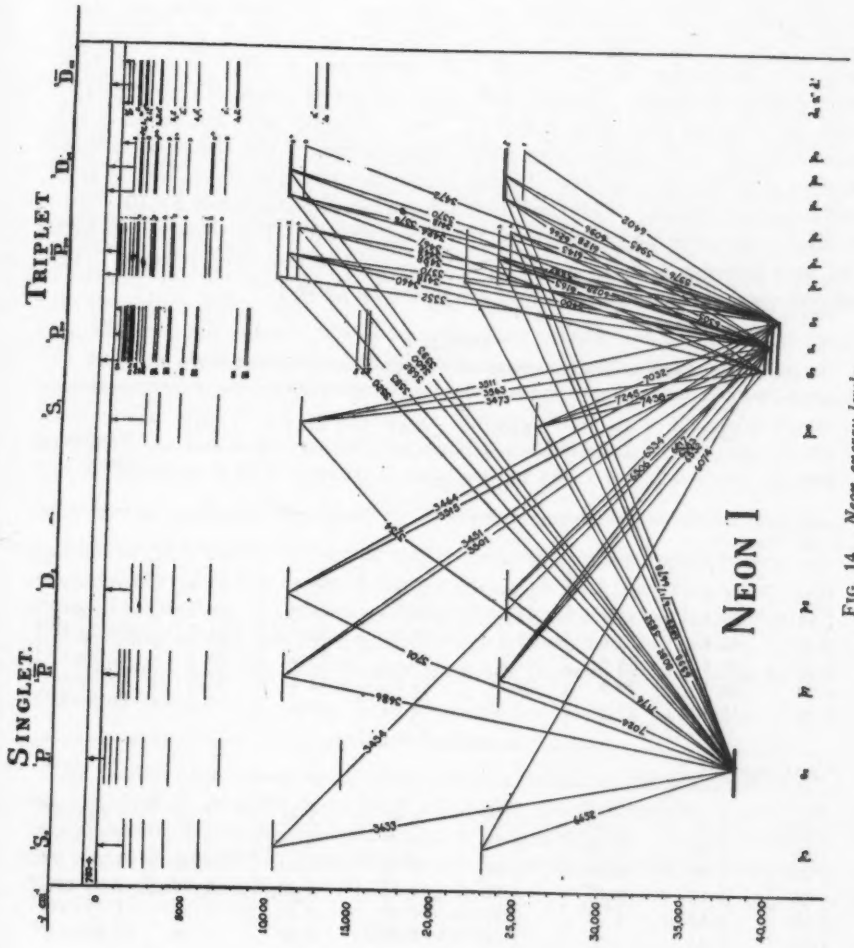

FIG. 14. *Neon energy levels.*

time difference in this case. The fact that log (D_1-D_2) plotted against t is a straight line justifies us in assigning a half-life value to the function D_1-D_2. The decay of (D_1-D_2) is plotted for six lines ending in the meta-stable states of neon (See Fig. 14): λ 6402, $(^3D_3-^3P_2)$; λ 6334, $(^1D_2-^3P_2)$; λ 6266, $(^3D_1-^3P_0)$; λ 6163, $(^3P_1-^3P_0)$; λ 6143, $(^3P_2-^3P_2)$; λ 5945, $(^3D_2-^3P_2)$. Maximum absorption of the order of 75% was obtained with these lines. For lines whose end states were $^3P_1$ or $^1P_1$ absorptions of more than 10% were never found, and absorption was only observable for very small time differences, so that no curves have been plotted for these. The time difference was set by means of the coupling C with an accuracy of at least 0.25×10^{-4} sec. Settings were not made at less than 5.0×10^{-4} sec. as for very short time differences the absorption should obey a more complicated law (1), due to the fact that the atoms are far from diffusion equilibrium. This time therefore (equals approximately 5×10^{-4} sec) is used as the arbitrary zero time of the curves shown. The variation of half-life with pressure is shown in the lower right hand section of each figure. The results upon which these are based are shown in Table I.

TABLE I

VALUES OF τ USED IN COMPUTATIONS OF VARIATION OF HALF-LIFE WITH PRESSURE

P, mm.	λ 6402	λ 6334	λ 6266	λ 6163	λ 6143	λ 5945	Number per cc.
	τ, sec. $\times10^{-4}$						
T, 80° Absolute							
1.33	18.6	41.5	34.9	29.4	40.2	42.3	16.2×10^{16}
1.69	36.5	52.4	43.5	43.8	48.0	49.7	20.6×10^{16}
2.56	45.4	72.8	54.7	58.9	53.5	83.0	31.2×10^{16}
3.30	50.1	66.8	67.0	68.7	53.2	96.5	40.2×10^{16}
4.55	40.1	29.9	29.4	28.0	32.5	37.8	55.5×10^{16}
6.58	35.4	13.1	7.30	5.65	19.4	18.5	80.0×10^{16}
T, 300° Absolute							
.500	3.92	1.43	1.47	1.86	3.66	2.53	1.62×10^{16}
.88	5.20	3.41	2.54	2.51	5.37	3.71	2.85×10^{16}
1.20	8.97	6.23	3.40	2.94	6.62	7.00	3.89×10^{16}
1.55	6.17	5.87	3.00	2.58	6.27	5.90	5.03×10^{16}
2.20	4.64	4.21	2.17	2.27	4.85	2.52	7.13×10^{16}
4.62	3.53	2.81	1.60	1.80	4.01	2.28	15.00×10^{16}

Discussion

It will be noted that the half-life value plotted against pressure gives very different curves for different transitions even though these end in the same lower state. Not only are the absolute magnitudes different but the shape of the curve seems different for different transitions. Thus we see that at liquid

air temperatures λ 6402 and λ 5945, both ending in the $^3P_2$ state, differ by a factor of almost two in the height of the maximum, a difference which is far beyond the limit of error. The lines λ 6402 and λ 6143 give curves which are of a similar nature and are not so different as to preclude their being actually the same. The line λ 6334 is definitely different from these and also from the line λ 5945. At this temperature λ 6163 and λ 6266, both ending in $^3P_0$, give curves which are in startlingly exact agreement. At room temperature on the other hand, λ 6402 and λ 5945 give curves of the same form though differing in magnitude, while the same is true for λ 6143 and λ 6334. The two pairs differ quite radically in the shape of the curve. λ 6163 and λ 6266 at room temperature differ somewhat but, as the error at these short half-life values is relatively large, it cannot be definitely said that they behave differently.

The error of the individual values of the points on the curves as stated in a previous paper is probably about 5%. This error is probably exceeded in the case of low values of the half-life, due, as stated above, to the occurrence of an error in the time settings which does not vary with their magnitude. Thus half-life values of less than 10×10^{-4} sec. are probably not accurate to closer than 10% of their value. It will be noted that these errors are far below the differences as shown between the various curves. Previous investigators (3) have suggested that a difference in half-width of the line might account for a difference in half-life as measured by different lines from the same state. Zemansky (4) has investigated this matter mathematically and has shown that a difference is to be expected, but that the effect will also show up as a departure of curves of $\log \frac{I}{I_0} = \frac{D_1 - D_2}{\gamma}$ plotted against time, from true exponentials. As shown by the plotted points, these measurements give exponential curves to the limits of their accuracy so that this explanation does not seem satisfactory. The alternative assumption that the "virtual oscillator" corresponding to each line has a real existence also seems rather radical, though less discordant with the more modern quantum theories than with the earlier ones.

Acknowledgment

The author wishes once again to express his thanks to the members of the technical staff of the laboratory, especially to Mr. Plaskett and Mr. Woodward whose assistance in the mechanical designing was invaluable. To Mr. Chappell also the author owes his thanks for much skilful work on the intricate glass flasks and discharge tubes.

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THE AIR AFTERGLOW AND ACTIVE NITROGEN[1]

By J. K. Robertson[2]

Abstract

In view of the lack of published photographs of the spectrum of the air afterglow, and of useful information which might be obtained relating to aurora discharges, the spectrum of this afterglow was photographed in the visible region. Excitation was accomplished by means of external electrodes used in connection with a Hartley oscillating circuit, a method which lends itself to the use of feeble excitations, and one, moreover, which does not seem to have been used hitherto. The spectrograms obtained show a continuous faintly banded region extending from below 4400 to 5000 or beyond, and a pure *continuum* in the yellow and red region with a distribution of intensity roughly corresponding to that shown by the yellow and red first positive nitrogen groups. Evidence is submitted that the part of the afterglow at the yellow-red end of the spectrum is due to nitrogen, not to oxygen, the source to which the continuous spectrum is generally ascribed.

In an attempt to obtain further information regarding the nature of the entity emitting the air afterglow, by the addition of mercury vapor to the glowing gas, it was found that the glow was at once destroyed.

Introduction

Although afterglows in gases subjected to an exciting discharge had been observed even before (2, 3, 11 p. 249, 19) the work of Lewis (14) and Strutt (23), comparatively little has been done on the spectrum of the air afterglow. It is particularly noteworthy that there seem to be no published photographs of this spectrum. Most observers agree in describing the spectrum as continuous. Thus, Strutt (23) records a continuous spectrum extending from 4200 to 6700 and "doubtless into the infra-red." In later work by the same investigator (24) in connection with his study of active nitrogen, a mixture of oxygen and nitrogen in the same proportions as in air gave a greenish-yellow glow with a continuous spectrum extending to 4300 on the short wave-length side, with the other limit uncertain. A somewhat similar observation has been made by Constantinides (1) and by Kaplan (8). Working with ordinary air, Hagenbach and Frey (5) obtained the characteristic yellow nitrogen afterglow, whereas Herzberg (6) in a nitrogen-oxygen mixture, as well as in air, by varying the conditions, was able to obtain either the yellow nitrogen afterglow or a green afterglow with continuous spectrum, extending from red to blue, which he ascribed to oxygen. Stöck (22), who worked with air, confirmed Herzberg's work. Somewhat similar is the observation of Bernard Lewis (12, 13) who, working with a mixture of oxygen and nitrogen could, under certain conditions, obtain the α bands of nitrogen with a blue continuous spectrum as background.

Majewska (16), following up the work of Pienkowski (20), who obtained with air a "diffuse broad band", states that the air afterglow spectrum is continuous in the visible region but shows lines in the ultra-violet.

Kaplan (8, 9), working with air, was able to obtain either a blue afterglow showing an active nitrogen spectrum, or, with altered conditions, a greenish-yellow glow with continuous spectrum.

[1] *Manuscript received August 25, 1932.*
Contribution from the Department of Physics, Queen's University, Kingston, Ontario.

[2] *Professor of Physics, Queen's University.*

Although there is considerable evidence that the air afterglow showing a continuous spectrum is due to oxygen, this has not been conclusively proved nor has the exact nature and origin of the "active species" been explained. It seemed worth while, therefore, particularly in view of the lack of published photographs and of the possible information which might be obtained relating to aurora discharges, to photograph carefully the air afterglow with a mode of excitation not hitherto used. Previous observers have made use of ordinary induction-coil discharges, transformer discharges, as well as those obtained with damped high-frequency currents. In the work described in this paper, the writer used continuous high-frequency currents, obtained by means of a three-electrode transmitting tube, a method which lends itself particularly well to the use of feeble excitations.

It was considered also that it might be possible to pass glowing air into a vapor such as mercury and by so doing obtain valuable information by methods already used in the study of active nitrogen.

Experimental

The experimental arrangement which is shown in Fig. 1 needs little explanation. The upper part of the diagram shows the connections for the well-known Hartley oscillating circuit involving a S.W. 2-A Mullard transmitting valve fed by a 1500-volt generator. Loosely coupled to the coil L_1 is a second coil L_2 attached to external electrodes, either in positions AA' or BB', between which the exciting discharge took place in air entering the apparatus through the control valve. No precautions were taken to dry or to purify the air in any way. To produce intermittent discharges a relay R was used which made and broke the grid-leak circuit by means of a commutator on the shaft of a motor rotating the sectored disk S. The discharge was flashed on and off about $3\frac{1}{3}$ times per second, the "on" period being about $\frac{1}{3}$ of the total time. For most of the work the wave-length of the high-frequency current was about 36 m.

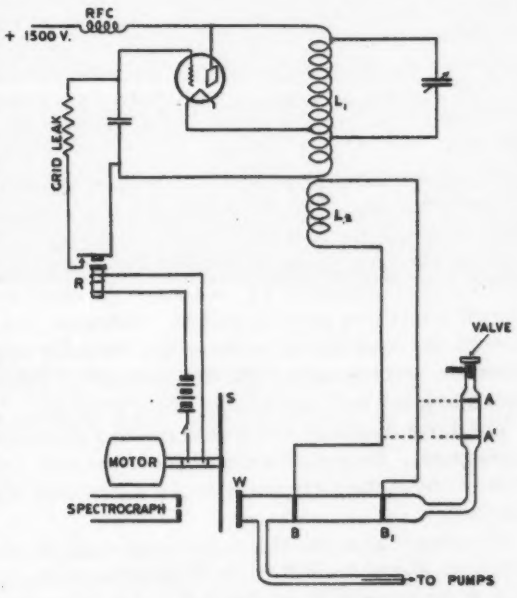


FIG. 1. *Diagram of circuit used to excite discharge in air and to photograph afterglow.*

The afterglow was photographed through the quartz window W with a

double-prism Ladd spectrograph which had previously been found extremely useful for sources of feeble intensity. Ilford special rapid panchromatic plates were used.

Some preliminary work was done to determine the most favorable conditions for obtaining a good afterglow. As the walls of the containing vessel undoubtedly have a marked influence on the afterglow (12), observations were made with three tubes of different sizes. The first was cylindrical and about 4 cm. in diameter; the second cylindrical, about 10 cm. in diameter; the third spherical, the radius being 13 or 14 cm. It was found that, although glows lasted somewhat longer in the largest vessel than in the smallest, they were of less intensity. Moreover, with both the largest vessel and that of intermediate size it was not so easy to obtain satisfactory glows. Most of the work, therefore, was carried out with the aid of cylindrical tubes 3 or 4 cm. in diameter.

To produce satisfactory afterglows, it was found that the air should not be stagnant. Indeed, during the period of several days which was necessary for a photograph of the spectrum, not only was air continually pumped through the apparatus (during actual exposure) but, in addition, each night, after shutting off the pumps, air was left in the system at atmospheric pressure. Somewhat similar is the observation of Majewska and Bernhardt (17), who found it necessary, in order to obtain a good afterglow, to put their tube in a "vorbereitet" condition by pumping air through or by washing out with air.

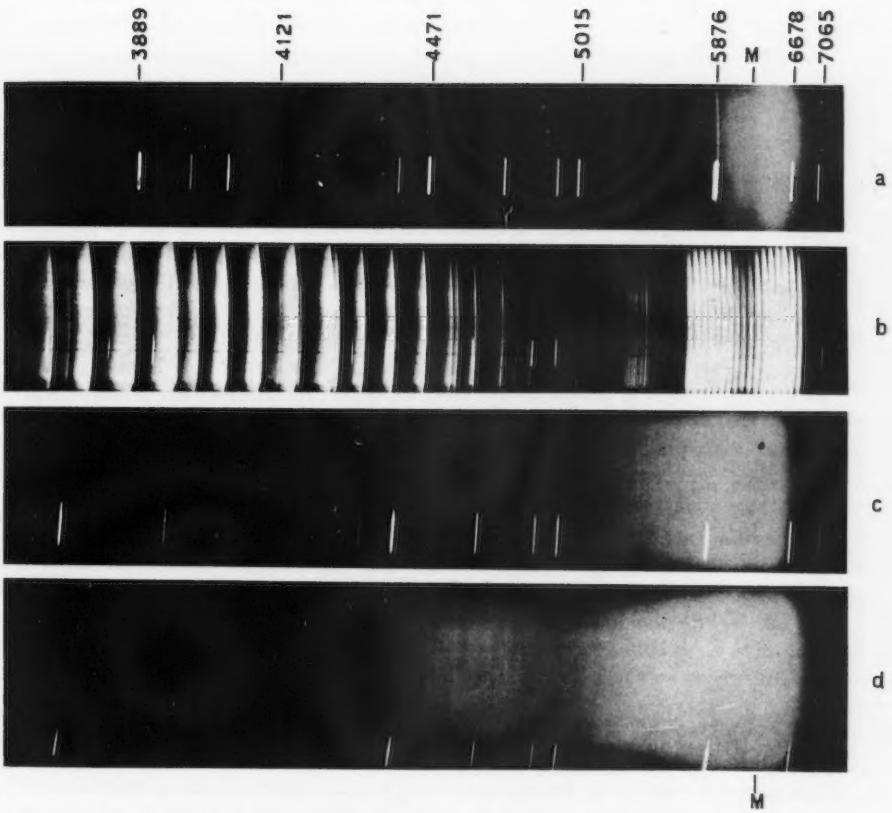
The intensity of the afterglow seemed, if anything, to improve with the length of time the discharge had been taking place, another indication, no doubt, of the sensitiveness of the afterglow to the conditions of the walls. Just what change is brought about in the surface by the electrical action, it is difficult to say, but that it is important is further emphasized by the statement of Kaplan (10) that active nitrogen was obtained by a new method which involved a two-weeks preliminary running of an uncondensed discharge in nitrogen.

Afterglows could be obtained at pressures ranging from about 1.25 mm. to as low as 0.03 mm. At the lower pressures, although the afterglow was sometimes of longer duration, it was less intense, and the predominantly greenish color changed to a somewhat whitish shade, *e.g.*, at pressures such as 0.12 mm. At the higher pressures, *e.g.*, 0.8 mm., the color was for the most part green, though sometimes greenish-yellow. However, one observation at 0.61 mm. showed the tendency to white which normally appeared at lower pressures. When the spectrograms *c* and *d* reproduced in Plate I were photographed the color was green and the pressure about 0.7 mm.

The direct discharge which gave rise to a good afterglow had a characteristic appearance. Between the electrodes it was pink, but on either side, a greenish glow of about the same shade as the subsequent afterglow extended for some distance.

For some reason, the direct discharge could be obtained more readily in the tube with electrodes BB' (tube of diameter about 2.4 cm. and length 42 cm.) than in the one with electrodes AA' (tube of diameter 4 cm. and length 15 cm.), although, with careful manipulation, discharges and satisfactory afterglows

PLATE I



Spectrum a: active nitrogen + calcium vapor; exposure, 3 hr.
Spectrum b: direct discharge in air; exposure 8 min.
Spectrum c: air afterglow; exposure 29 hr.
Spectrum d: air afterglow; exposure 29 hr.

could be obtained in the latter. Indeed, under favorable conditions with this tube, the green extension of the direct discharge extended so that it filled the tube with the quartz window, where, of course, it persisted on the removal of the exciting potential. As the afterglow was of short duration, never lasting for longer than seven seconds, and as such satisfactory glows were obtained when electrodes AA' were used, the spectrograms were taken with the direct discharge in the tube to which they were attached.

Results

In the accompanying plate, Spectrum *b* is that of the direct discharge. The marked intensity of the first and second positive bands of nitrogen will be noted. Both *c* and *d* are spectra of the afterglow, the exposure for each being 29 hr. It may not be amiss to state that between the exposures the apparatus was partly dismantled and a new tube installed in place of the old one in which the discharge had been taking place. It will be noted that both spectra show a continuous faintly banded region extending from below 4400 to 5000 or beyond, as well as a *continuum* beginning in the region corresponding to the green group of the first positive band system and extending to the long wavelength side of the red group. If the detail of individual bands be neglected, the intensity distribution in this truly continuous region is closely parallel to that shown by the green, yellow and red first positive groups in the direct discharge. Although the reproduction does not show it well, in both *c* and *d* there is a pronounced minimum corresponding in position to the region of minimum intensity between the yellow and red groups in the direct discharge.

(At the lower edge of Spectrum *d*, the presence of second positive bands as well as the typical structure of the red first positive group will be noticed. This is undoubtedly due to a faulty adjustment of the rotating sector which allowed a trace of light from the direct discharge to enter one end of the slit of the spectrograph. No sign of either second positive bands or of structure in the red end of the afterglow appears in the upper half of this spectrum, nor is there any sign of either in Spectrum *c*.)

Before discussing the above spectra, it is desirable to refer briefly to the results obtained when mercury vapor was added to the gas in the tubes. For this purpose a small amount of mercury was inserted in a side tube attached to the long tube with the quartz window. Before warming the mercury, the direct discharge showed the characteristic pink color with the diffuse green extension. On heating the mercury the green glow disappeared entirely and suddenly. Difficulty was subsequently experienced in obtaining a good afterglow even in the presence of mercury at room temperature. By cooling the side arm tube with solid carbon dioxide most of the mercury could be collected in that place, but even under those conditions, after the discharge had been running for an hour, the afterglow was of less intensity than at the outset. It may be concluded, therefore, that the presence of mercury vapor in appreciable amounts inhibits or destroys this type of afterglow.

Discussion

Although Strutt in his early work on the air afterglow ascribed the origin to the combination of ozone with nitric oxide, and although recent observers have stated that the green afterglow with continuous spectrum is due to oxygen, it seems to the writer that the origin of that part of the afterglow giving rise to the yellow and red end of the spectrum must be related to nitrogen. The evidence for this is found in the fact that the spectrum of the exciting discharge is that of nitrogen, as shown in Spectrum *b*, but most of all, in the information yielded by an examination of Spectrum *a*. This is a reproduction of one of several plates taken in this laboratory some four years ago by J. H. Findlay (4) in connection with his investigation of certain metallic spectra excited by active nitrogen. It will be noted that, allowing for differences in exposure, there is almost an exact agreement between Spectrum *a* and Spectra *c* and *d* in so far as the yellow-red end is concerned. Note, for example, the position of the region of minimum intensity (marked *M*) in the spectra. In Spectra *c* and *d* the yellow and red bands seem to be truly continuous but a close examination of the red band in Spectrum *a* reveals the presence of a structure which seems to be identical with that of the red group of the first positive bands of nitrogen.

Now, in Findlay's work, streaming active nitrogen, generated by a strong electrodeless discharge in nitrogen (not air) was passed into a tube in which, for Spectrum *a*, metallic calcium was strongly heated. The resulting spectrum, reproduced in *a*, showed sodium (a calcium impurity or perhaps from the heated Pyrex) lines and the yellow and red apparently continuous region to which attention has been called. This same bit of apparently continuous spectrum was observed by Findlay on another calcium plate as well as on one obtained when mercury vapor was added to active nitrogen. A six-hour exposure on the active nitrogen itself, however, showed only the α collection of first positive nitrogen bands. There seems no doubt, therefore, that that part of the air afterglow giving rise to the yellow and red end of Spectra *c* and *d* is due to nitrogen, not to oxygen. There is, of course, the possibility that oxygen might have been given off by the heated calcium and mercury and excited by active nitrogen but, in an exposure lasting several hours, one would think that after the first heating, most of the occluded oxygen would have been removed. Oxygen bands may be obtained in the red-yellow region but, according to the work of Lockrow (15), the potential necessary to excite these is over 20 volts. As active nitrogen has given rise to no lines requiring an excitation potential greater than 9.5 volts the yellow-red described in this paper could have no connection with these oxygen bands.

That active nitrogen can be obtained by discharges in air has been established by the work of Herzberg (6), Kaplan (8), and Mukherji (18), but the glow observed does not seem to be of the same type as that described in this paper. Certainly that obtained by Kaplan is different, for he states that his afterglow showed typical α bands of nitrogen as well as the β and α bands of nitric oxide.

It seems to the writer that there is evidence in favor of the view that this yellow-red afterglow results from a modified, perturbed emission of nitrogen

bands brought about by the action of some agency on excited nitrogen molecules. In Findlay's plate the perturbation is not enough to completely remove the band structure; in the air afterglow, it is more pronounced and the structure disappears. The phenomenon is probably not unrelated to the modification in the distribution of intensity in the α group of active nitrogen, observed by Rayleigh (21) and by Herzberg (7). Rayleigh obtained a marked shifting of intensity to the red group by the addition of helium, whereas Herzberg, by cooling with liquid air, observed a shift in the opposite direction.

In Findlay's work it will be recalled, the continuous band was obtained only when active nitrogen encountered a foreign vapor. Just what the process is that perturbs the vibrational levels is difficult to see, but the evidence seems to favor strongly some such process rather than the ascribing of the appearance to a reaction between ozone and nitric oxide, or to a recombination spectrum of oxygen.

It is probable that the remainder of the afterglow spectrum shown in *c* and *d* *i.e.*, the blue-green portion with faint structure, is due to oxygen, as there is no trace of this part in Spectrum *a*. The writer had hoped to photograph the afterglow from pure oxygen but circumstances prevented him doing so, and other work will not permit this being done immediately.

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